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(54) Title: TOTIPOTENT HEMATOPOIETIC STEM CELL RECEPTORS AND THEIR LIGANDS

(57) Abstract

Isolated mammalian nucleic acid molecules encoding receptor protein tyrosine kinases expressed in primitive hematopoietic cells and not expressed in mature hematopoietic cells are provided. Also included are the receptors encoded by such nucleic acid molecules; the nucleic acid molecules encoding receptor protein tyrosine kinases having the sequences shown in Figure 1 (flk-2) and Figure 2 (flk-1); the receptor protein tyrosine kinases having the amino acid sequences shown in Figure 1 (flk-2) and Figure 2 (flk-1); ligands for the receptors; nucleic acid sequences that encode the ligands; and methods of stimulating the proliferation and/or differentiation of primitive mammalian hematopoietic stem cells comprising contacting the stem cells with a ligand that binds to a receptor protein tyrosine kinase expressed in primitive mammalian hematopoietic cells and not expressed in mature hematopoietic cells.

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TOTIPOTENT HEMATOPOIETIC STEM CELL RECEPTORS AND THEIR LIGANDS

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FIELD OF THE INVENTION

The present invention relates to hematopoietic stem cell receptors, ligands for such receptors, and nucleic acid molecules encoding such receptors and ligands.

BACKGROUND OF THE INVENTION

The mammalian hematopoietic system comprises red and white blood cells. These cells are the mature cells that result from more primitive lineage-restricted cells. The cells of the hematopoietic system have been reviewed by Dexter and Spooncer in the Annual Review of Cell Biology 3, 423-441 (1987).

The red blood cells, or erythrocytes, result from primitive cells referred to by Dexter and Spooncer as erythroid burst-forming units (BFU-E). The immediate progeny of the erythroid burst-forming units are called erythroid colony-forming units (CFU-E).

The white blood cells contain the mature cells of the lymphoid and myeloid systems. The lymphoid cells include B lymphocytes and T lymphocytes. The B and T lymphocytes result from earlier progenitor cells referred to by Dexter and Spooncer as preT and preB cells.

The myeloid system comprises a number of cells including granulocytes, platelets, monocytes, macrophages, and

megakaryocytes. The granulocytes are further divided into neutrophils, eosinophils, basophils and mast cells.

Each of the mature hematopoietic cells are specialized for specific functions. For example, erythrocytes are responsible for oxygen and carbon dioxide transport. T and B lymphocytes are responsible for cell-and antibody-mediated immune responses, respectively. Platelets are involved in blood clotting. Granulocytes and macrophages act generally as scavengers and accessory cells in the immune response against invading organisms and their by-products.

At the center of the hematopoietic system lie one or more totipotent hematopoietic stem cells, which undergo a series of differentiation steps leading to increasingly lineage-restricted progenitor cells. The more mature progenitor cells are restricted to producing one or two lineages. Some examples of lineage-restricted progenitor cells mentioned by Dexter and Spooncer include granulocyte/macrophage colony-forming cells (GM-CFC), megakaryocyte colony-forming cells (Meg-CFC), eosinophil colony-forming cells (Eos-CFC), and basophil colony-forming cells (Bas-CFC). Other examples of progenitor cells are discussed above.

The hematopoietic system functions by means of a precisely controlled production of the various mature lineages. The totipotent stem cell possesses the ability both to self renew and to differentiate into committed progenitors for all hematopoietic lineages. These most primitive of hematopoietic cells are both necessary and sufficient for the complete and permanent hematopoietic reconstitution of a radiation-ablated hematopoietic system in mammals. The ability of stem cells to reconstitute the entire hematopoietic system is the basis of bone marrow transplant therapy.

It is known that growth factors play an important role in the development and operation of the mammalian

hematopoietic system. The role of growth factors is complex, however, and not well understood at the present time. One reason for the uncertainty is that much of what is known about hematopoietic growth factors results from in vitro experiments. Such experiments do not necessarily reflect in vivo realities.

In addition, in vitro hematopoiesis can be established in the absence of added growth factors, provided that marrow stromal cells are added to the medium. The relationship between stromal cells and hematopoietic growth factors in vivo is not understood. Nevertheless, hematopoietic growth factors have been shown to be highly active in vivo.

From what is known about them, hematopoietic growth factors appear to exhibit a spectrum of activities. At one end of the spectrum are growth factors such as erythropoietin, which is believed to promote proliferation only of mature erythroid progenitor cells. In the middle of the spectrum are growth factors such as IL-3, which is believed to facilitate the growth and development of early stem cells as well as of numerous progenitor cells. Some examples of progenitor cells induced by IL-3 include those restricted to the granulocyte/macrophage, eosinophil, megakaryocyte, erythroid and mast cell lineages.

At the other end of the spectrum is the hematopoietic growth factor that, along with the corresponding receptor, was discussed in a series of articles in the October 5, 1990 edition of Cell. The receptor is the product of the W locus, c-kit, which is a member of the class of receptor protein tyrosine kinases. The ligand for c-kit, which is referred to by various names such as stem cell factor (SCF) and mast cell growth factor (MGF), is believed to be essential for the development of early hematopoietic stem cells and cells restricted to the erythroid and mast cell lineages in mice; see, for example, Copeland et al., Cell 63, 175-183 (1990).

It appears, therefore, that there are growth factors

that exclusively affect mature cells. There also appear to be growth factors that affect both mature cells and stem cells. The growth factors that affect both types of cells may affect a small number or a large number of mature cells.

There further appears to be an inverse relationship between the ability of a growth factor to affect mature cells and the ability of the growth factor to affect stem cells. For example, the c-kit ligand, which stimulates a small number of mature cells, is believed to be more important in the renewal and development of stem cells then is IL-3, which is reported to stimulate proliferation of many mature cells (see above).

Prior to the present specification, there have been no reports of growth factors that exclusively stimulate stem cells in the absence of an effect on mature cells. The discovery of such growth factors would be of particular significance.

As mentioned above, c-kit is a protein tyrosine kinase (pTK). It is becoming increasingly apparent that the protein tyrosine kinases play an important role as cellular receptors for hematopoietic growth factors. Other receptor pTKs include the receptors of colony stimulating factor 1 (CSF-1) and PDGF.

The pTK family can be recognized by the presence of several conserved amino acid regions in the catalytic domain. These conserved regions are summarized by Hanks et al. in Science <u>241</u>, 42-52 (1988), see Figure 1 starting on page 46 and by Wilks in Proc. Natl. Acad. Sci. USA <u>86</u>, 1603-1607 (1989), see Figure 2 on page 1605.

Additional protein tyrosine kinases that represent hematopoietic growth factor receptors are needed in order more effectively to stimulate the self-renewal of the totipotent hematopoietic stem cell and to stimulate the development of all cells of the hematopoietic system both <u>in</u>

vitro and *in vivo*. Novel hematopoietic growth factor receptors that are present only on primitive stem cells, but are not present on mature progenitor cells, are particularly desired. Ligands for the novel receptors are also desirable to act as hematopoietic growth factors. Nucleic acid sequences encoding the receptors and ligands are needed to produce recombinant receptors and ligands.

SUMMARY OF THE INVENTION

These and other objectives as will be apparent to those with ordinary skill in the art have been met by providing isolated mammalian nucleic acid molecules encoding receptor protein tyrosine kinases expressed in primitive hematopoietic cells and not expressed in mature hematopoietic cells. Also included are the receptors encoded by such nucleic acid molecules; the nucleic acid molecules encoding receptor protein tyrosine kinases having the sequences shown in Figure 1 (flk-2) and Figure 2 (flk-1); the receptor protein tyrosine kinases having the amino acid sequences shown in Figure 1 (flk-2) and Figure 2 (flk-1); ligands for the receptors; nucleic acid sequences that encode the ligands; and methods of stimulating the proliferation of primitive mammalian hematopoietic stem cells comprising contacting the stem cells with a ligand that binds to a receptor protein tyrosine kinase expressed in primitive mammalian hematopoietic cells and not expressed in mature hematopoietic cells.

DESCRIPTION OF THE FIGURES

Figure 1a.1-1a.3 shows the cDNA and amino acid sequences of murine flk-2. The amino acid residues occur directly below the nucleotides in the open reading frame. Amino acids 1-27 constitute the hydrophobic leader sequence. Amino acids 28-544 constitute the extracellular receptor domain. Amino acids 545-564 constitute the transmembrane region. The remainder of the amino acids constitute the intracellular catalytic domain. The following amino acid residues in the intracellular domain are catalytic sub-domains identified by

Hanks (see above): 545-564, 618-623, 811-819, 832-834, 857-862, 872-878. The sequence at residues 709-785 is a signature sequence characteristic of flk-2. The protein tyrosine kinases generally have a signature sequence in this region.

Figure 1b shows the cDNA and amino acid sequences of a portion of human flk-2 from the extracellular domain. Amino acids 1-110 of the human flk-2 correspond to amino acids 43-152 of murine flk-2.

Figure 1c shows the cDNA and amino acid sequences of a portion of human flk-2 from the intracellular (kinase) domain. Amino acids 1-94 of the human flk-2 correspond to amino acids 751-849 of murine flk-2.

Figure 2-2.3 shows the cDNA and amino acid sequences of flk-1. Amino acid residue 763-784 constitute the transmembrane region of flk-1.

Figure 3 shows the time response of binding between a murine stromal cell line (2018) and APtag-flk-2 as well as APtag-flk-1. APtag without receptor (SEAP) is used as a control. See Example 8.

Figure 4 shows the dose response of binding between stromal cells (2018) and APtag-flk-2 as well as APtag-flk-1. APtag without receptor (SEAP) is used as a control. See Example 8.

DETAILED DESCRIPTION OF THE INVENTION

Receptors

In one embodiment, the invention relates to an isolated mammalian nucleic acid molecule encoding a receptor protein tyrosine kinase expressed in primitive mammalian hematopoietic cells and not expressed in mature hematopoietic cells.

The nucleic acid molecule may be a DNA, cDNA, or RNA molecule. The mammal in which the nucleic acid molecule exists may be any mammal, such as a mouse, rat, rabbit, or human.

The nucleic acid molecule encodes a protein tyrosine kinase (pTK). Members of the pTK family can be recognized by the conserved amino acid regions in the catalytic domains. Examples of pTK consensus sequences have been provided by Hanks et al. in Science <u>241</u>, 42-52 (1988); see especially Figure 1 starting on page 46 and by Wilks in Proc. Natl. Acad. Sci. USA <u>86</u>, 1603-1607 (1989); see especially Figure 2 on page 1605. A methionine residue at position 205 in the conserved sequence WMAPES is characteristic of pTK's that are receptors.

The Hanks et al article identifies eleven catalytic sub-domains containing pTK consensus residues and sequences. The pTKs of the present invention will have most or all of these consensus residues and sequences.

Some particularly strongly conserved residues and sequences are shown in Table 1.

TABLE 1

Conserved Residues and Sequences in pTKs[1]

Position[2]	Residue or Sequence	Catalytic Domain
50	G	I
52	G	I
57	V	I
70	A	II
72	K	II
91	E	III
166	D	VI
171	N	VI
184-186	DFG	VII
208	E	VIII
220	D	IX
225	G	IX
280	R	XI

1. See Hanks et al., Science <u>241</u>, 42-52 (1988)
2. Adjusted in accordance with Hanks et al., Id.

A pTK of the invention may contain all thirteen of these highly conserved residues and sequences. As a result of natural or synthetic mutations, the pTKs of the invention may contain fewer than all thirteen strongly conserved residues and sequences, such as 11, 9, or 7 such sequences.

The receptors of the invention generally belong to the same class of pTK sequences that c-kit belongs to. It has surprisingly been discovered, however, that a new functional class of receptor pTKs exists. The new functional class of receptor pTKs is expressed in primitive hematopoietic cells, but not expressed in mature hematopoietic cells.

For the purpose of this specification, a primitive hematopoietic cell is totipotent, i.e. capable of reconstituting all hematopoietic blood cells *in vivo*. A mature hematopoietic cell is non-self-renewing, and has limited proliferative capacity - i.e., a limited ability to give rise to multiple lineages. Mature hematopoietic cells, for the purposes of this specification, are generally capable of giving rise to only one or two lineages *in vitro* or *in vivo*.

It should be understood that the hematopoietic system is complex, and contains many intermediate cells between the primitive totipotent hematopoietic stem cell and the totally committed mature hematopoietic cells defined above. As the stem cell develops into increasingly mature, lineage-restricted cells, it gradually loses its capacity for self-renewal.

The receptors of the present invention may and may not be expressed in these intermediate cells. The necessary and sufficient condition that defines members of the new class of receptors is that they are present in the primitive, totipotent stem cell or cells, and not in mature cells restricted only to one or, at most, two lineages.

An example of a member of the new class of receptor pTKs

is called fetal liver kinase 2 (flk-2) after the organ in which it was found. There is approximately 1 totipotent stem cell per 10^4 cells in mid-gestation (day 14) fetal liver in mice. In addition to fetal liver, flk-2 is also expressed in fetal spleen, fetal thymus, adult brain, and adult marrow.

For example, flk-2 is expressed in individual multipotential CFU-Blast colonies capable of generating numerous multilineage colonies upon replating. It is likely, therefore, that flk-2 is expressed in the entire primitive (i.e. self-renewing) portion of the hematopoietic hierarchy. This discovery is consistent with flk-2 being important in transducing putative self-renewal signals from the environment.

It is particularly relevant that the expression of flk-2 mRNA occurs in the most primitive thymocyte subset. Even in two closely linked immature subsets that differ in expression of the IL-2 receptor, flk-2 expression segregates to the more primitive subset lacking an IL-2 receptor. The earliest thymocyte subset is believed to be uncommitted. Therefore, the thymocytes expressing flk-2 may be multipotential. flk-2 is the first receptor tyrosine kinase known to be expressed in the T-lymphoid lineage.

The fetal liver mRNA migrates relative to 285 and 185 ribosomal bands on formaldehyde agarose gels at approximately 3.5 kb while the brain message is considerably larger. In adult tissues, flk-2 m-RNA from both brain and bone marrow migrated at approximately 3.5 kb.

A second pTK receptor is also included in the present invention. This second receptor, which is called fetal liver kinase 1 (flk-1), is not a member of the same class of receptors as flk-2, since flk-1 may be found in some more mature hematopoietic cells. The amino acid sequence of flk-1 is given in Figure 2.

The present invention includes the flk-1 receptor as

well as DNA, cDNA and RNA encoding flk-1. The DNA sequence of flk-1 is also given in Figure 2. Flk-1 may be found in the same organs as flk-2, as well as in fetal brain, stomach, kidney, lung, heart and intestine; and in adult kidney, heart, spleen, lung, muscle, and lymph nodes.

The receptor protein tyrosine kinases of the invention are known to be divided into easily found domains. The DNA sequence corresponding to the pTKs encode, starting at their 5′-ends, a hydrophobic leader sequence followed by a hydrophilic extracellular domain, which binds to, and is activated by, a specific ligand. Immediately downstream from the extracellular receptor domain, is a hydrophobic transmembrane region. The transmembrane region is immediately followed by a basic catalytic domain, which may easily be identified by reference to the Hanks et al. and Wilks articles discussed above.

The present invention includes the extracellular receptor domain lacking the transmembrane region and catalytic domain. Preferably, the hydrophobic leader sequence is also removed from the extracellular domain. In the case of flk-2, the hydrophobic leader sequence includes amino acids 1-27.

These regions and domains may easily be visually identified by those having ordinary skill in the art by reviewing the amino acid sequence in a suspected pTK and comparing it to known pTKs. For example, referring to Figure 1a, the transmembrane region of flk-2, which separates the extracellular receptor domain from the catalytic domain, is encoded by nucleotides 1663 (T) to 1722 (C). These nucleotides correspond to amino acid residues 545 (Phe) to 564 (Cys). The amino acid sequence between the transmembrane region and the catalytic sub-domain (amino acids 618-623) identified by Hanks et al. as sub-domain I (i.e., GXGXXG) is characteristic of receptor protein tyrosine kinases.

The extracellular domain may also be identified through

commonly recognized criteria of extracellular amino acid sequences. The determination of appropriate criteria is known to those skilled in the art, and has been described, for example, by Hopp et al, Proc. Nat'l Acad. Sci. USA 78, 3824-3828 (1981); Kyte et al, J. Mol. Biol. 157, 105-132 (1982); Emini, J. Virol. 55, 836-839 (1985); Jameson et al, CA BIOS 4, 181-186 (1988); and Karplus et al, Naturwissenschaften 72, 212-213 (1985). Amino acid domains predicted by these criteria to be surface exposed characteristic of extracellular domains.

As will be discussed in more detail below, the nucleic acid molecules that encode the receptors of the invention may be inserted into known vectors for use in standard recombinant DNA techniques. Standard recombinant DNA techniques are those such as are described in Sambrook et al., "Molecular Cloning," Second Edition, Cold Spring Harbor Laboratory Press (1987) and by Ausubel et al., Eds, "Current Protocols in Molecular Biology," Green Publishing Associates and Wiley-Interscience, New York (1987). The vectors may be circular (i.e. plasmids) or non-circular. Standard vectors are available for cloning and expression in a host. The host may be prokaryotic or eucaryotic. Prokaryotic hosts are preferably E. coli. Preferred eucaryotic hosts include yeast, insect and mammalian cells. Preferred mammalian cells include, for example, CHO, COS and human cells.

Ligands

The invention also includes ligands that bind to the receptor pTKs of the invention. In addition to binding, the ligands stimulate the proliferation of additional primitive stem cells, differentiation into more mature progenitor cells, or both.

The ligand may be a growth factor that occurs naturally in a mammal, preferably the same mammal that produces the corresponding receptor. The growth factor may be isolated and purified, or be present on the surface of an isolated

population of cells, such as stromal cells.

The ligand may also be a molecule that does not occur naturally in a mammal. For example, antibodies, preferably monoclonal, raised against the receptors of the invention or against anti-ligand antibodies mimic the shape of, and act as, ligands if they constitute the negative image of the receptor or anti-ligand antibody binding site. The ligand may also be a non-protein molecule that acts as a ligand when it binds to, or otherwise comes into contact with, the receptor.

In another embodiment, nucleic acid molecules encoding the ligands of the invention are provided. The nucleic acid molecule may be RNA, DNA or cDNA.

Stimulating Proliferation of Stem Cells

The invention also includes a method of stimulating the proliferation and/or differentiation of primitive mammalian hematopoietic stem cells as defined above. The method comprises contacting the stem cells with a ligand in accordance with the present invention. The stimulation of proliferation and/or differentiation may occur in vitro or in vivo.

The ability of a ligand according to the invention to stimulate proliferation of stem cells in vitro and in vivo has important therapeutic applications. Such applications include treating mammals, including humans, whose primitive stem cells do not sufficiently undergo self-renewal. Example of such medical problems include those that occur when defects in hematopoietic stem cells or their related growth factors depress the number of white blood cells. Examples of such medical problems include anemia, such as macrocytic and aplastic anemia. Bone marrow damage resulting from cancer chemotherapy and radiation is another example of a medical problem that would be helped by the stem cell factors of the invention.

Functional Equivalents

The invention includes functional equivalents of the pTK receptors, receptor domains, and ligands described above as well as of the nucleic acid sequences encoding them. A protein is considered a functional equivalent of another protein for a specific function if the equivalent protein is immunologically cross-reactive with, and has the same function as, the receptors and ligands of the invention. The equivalent may, for example, be a fragment of the protein, or a substitution, addition or deletion mutant of the protein.

For example, it is possible to substitute amino acids in a sequence with equivalent amino acids. Groups of amino acids known normally to be equivalent are:

(a) Ala(A) Ser(S) Thr(T) Pro(P) Gly(G);
(b) Asn(N) Asp(D) Glu(E) Gln(Q);
(c) His(H) Arg(R) Lys(K);
(d) Met(M) Leu(L) Ile(I) Val(V); and
(e) Phe(F) Tyr(Y) Trp(W).

Substitutions, additions and/or deletions in the receptors and ligands may be made as long as the resulting equivalent receptors and ligands are immunologically cross reactive with, and have the same function as, the native receptors and ligands.

The equivalent receptors and ligands will normally have substantially the same amino acid sequence as the native receptors and ligands. An amino acid sequence that is substantially the same as another sequence, but that differs from the other sequence by means of one or more substitutions, additions and/or deletions is considered to be an equivalent sequence. Preferably, less than 25%, more preferably less than 10%, and most preferably less than 5% of the number of amino acid residues in the amino acid sequence of the native receptors and ligands are substituted for, added to, or deleted from.

Equivalent nucleic acid molecules include nucleic acid sequences that encode equivalent receptors and ligands as defined above. Equivalent nucleic acid molecules also include nucleic acid sequences that differ from native nucleic acid sequences in ways that do not affect the corresponding amino acid sequences.

ISOLATION OF NUCLEIC ACID MOLECULES AND PROTEINS

Isolation of Nucleic Acid Molecules Encoding Receptors

In order to produce nucleic acid molecules encoding mammalian stem cell receptors, a source of stem cells is provided. Suitable sources include fetal liver, spleen, or thymus cells or adult marrow or brain cells.

For example, suitable mouse fetal liver cells may be obtained at day 14 of gestation. Mouse fetal thymus cells may be obtained at day 14-18, preferably day 15, of gestation. Suitable fetal cells of other mammals are obtained at gestation times corresponding to those of mouse.

Total RNA is prepared by standard procedures from stem cell receptor-containing tissue. The total RNA is used to direct cDNA synthesis. Standard methods for isolating RNA and synthesizing cDNA are provided in standard manuals of molecular biology such as, for example, in Sambrook et al., "Molecular Cloning," Second Edition, Cold Spring Harbor Laboratory Press (1987) and in Ausubel et al., (Eds), "Current Protocols in Molecular Biology," Greene Associates/Wiley Interscience, New York (1990).

The cDNA of the receptors is amplified by known methods. For example, the cDNA may be used as a template for amplification by polymerase chain reaction (PCR); see Saiki et al., Science, 239, 487 (1988) or Mullis et al., U.S. patent 4,683,195. The sequences of the oligonucleotide primers for the PCR amplification are derived from the sequences of known receptors, such as from the sequences

given in Figures 1 and 2 for flk-2 and flk-1, respectively, preferably from flk-2. The oligonucleotides are synthesized by methods known in the art. Suitable methods include those described by Caruthers in Science 230, 281-285 (1985).

In order to isolate the entire protein-coding regions for the receptors of the invention, the upstream oligonucleotide is complementary to the sequence at the 5′ end, preferably encompassing the ATG start codon and at least 5-10 nucleotides upstream of the start codon. The downstream oligonucleotide is complementary to the sequence at the 3′ end, optionally encompassing the stop codon. A mixture of upstream and downstream oligonucleotides are used in the PCR amplification. The conditions are optimized for each particular primer pair according to standard procedures. The PCR product is analyzed by electrophoresis for the correct size cDNA corresponding to the sequence between the primers.

Alternatively, the coding region may be amplified in two or more overlapping fragments. The overlapping fragments are designed to include a restriction site permitting the assembly of the intact cDNA from the fragments.

The amplified DNA encoding the receptors of the invention may be replicated in a wide variety of cloning vectors in a wide variety of host cells. The host cell may be prokaryotic or eukaryotic. The DNA may be obtained from natural sources and, optionally, modified, or may be synthesized in whole or in part.

The vector into which the DNA is spliced may comprise segments of chromosomal, non-chromosomal and synthetic DNA sequences. Some suitable prokaryotic cloning vectors include plasmids from E. coli, such as colE1, pCR1, pBR322, pMB9, pUC, pKSM, and RP4. Prokaryotic vectors also include derivatives of phage DNA such as M13 and other filamentous single-stranded DNA phages.

Isolation of Receptors

DNA encoding the receptors of the invention are inserted into a suitable vector and expressed in a suitable prokaryotic or eucaryotic host. Vectors for expressing proteins in bacteria, especially E.coli, are known. Such vectors include the PATH vectors described by Dieckmann and Tzagoloff in J. Biol. Chem. 260, 1513-1520 (1985). These vectors contain DNA sequences that encode anthranilate synthetase (TrpE) followed by a polylinker at the carboxy terminus. Other expression vector systems are based on beta-galactosidase (pEX); lambda P_L; maltose binding protein (pMAL); and glutathione S-transferase (pGST) - see Gene 67, 31 (1988) and Peptide Research 3, 167 (1990).

Vectors useful in yeast are available. A suitable example is the 2μ plasmid.

Suitable vectors for use in mammalian cells are also known. Such vectors include well-known derivatives of SV-40, adenovirus, retrovirus-derived DNA sequences and vectors derived from combination of plasmids and phage DNA.

Further eukaryotic expression vectors are known in the art (e.g., P.J. Southern and P. Berg, J. Mol. Appl. Genet. 1, 327-341 (1982); S. Subramani et al, Mol. Cell. Biol. 1, 854-864 (1981); R.J. Kaufmann and P.A. Sharp, "Amplification And Expression Of Sequences Cotransfected with A Modular Dihydrofolate Reductase Complementary DNA Gene," J. Mol. Biol. 159, 601-621 (1982); R.J. Kaufmann and P.A. Sharp, Mol. Cell. Biol. 159, 601-664 (1982); S.I. Scahill et al, "Expression And Characterization Of The Product Of A Human Immune Interferon DNA Gene In Chinese Hamster Ovary Cells," Proc. Natl. Acad. Sci. USA 80, 4654-4659 (1983); G. Urlaub and L.A. Chasin, Proc. Natl. Acad. Sci. USA 77, 4216-4220, (1980).

The expression vectors useful in the present invention contain at least one expression control sequence that is

operatively linked to the DNA sequence or fragment to be expressed. The control sequence is inserted in the vector in order to control and to regulate the expression of the cloned DNA sequence. Examples of useful expression control sequences are the lac system, the trp system, the tac system, the trc system, major operator and promoter regions of phage lambda, the control region of fd coat protein, the glycolytic promoters of yeast, e.g., the promoter for 3-phosphoglycerate kinase, the promoters of yeast acid phosphatase, e.g., Pho5, the promoters of the yeast alpha-mating factors, and promoters derived from polyoma, adenovirus, retrovirus, and simian virus, e.g., the early and late promoters or SV40, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells and their viruses or combinations thereof.

Vectors containing the receptor-encoding DNA and control signals are inserted into a host cell for expression of the receptor. Some useful expression host cells include well-known prokaryotic and eukaryotic cells. Some suitable prokaryotic hosts include, for example, E. coli, such as E. coli SG-936, E. coli HB 101, E. coli W3110, E. coli X1776, E. coli X2282, E. coli DHI, and E. coli MRC1, Pseudomonas, Bacillus, such as Bacillus subtilis, and Streptomyces. Suitable eukaryotic cells include yeast and other fungi, insect, animal cells, such as COS cells and CHO cells, human cells and plant cells in tissue culture.

The human homologs of the mouse receptors described above are isolated by a similar strategy. RNA encoding the receptors are obtained from a source of human cells enriched for primitive stem cells. Suitable human cells include fetal spleen, thymus and liver cells, and umbilical cord blood as well as adult brain and bone marrow cells. The human fetal cells are preferably obtained on the day of gestation corresponding to mid-gestation in mice. The amino acid sequences of the human flk receptors as well as of the nucleic acid sequences encoding them are homologous to the amino acid and nucleotide sequences of the mouse receptors.

In the present specification, the sequence of a first protein, such as a receptor or a ligand, or of a nucleic acid molecule that encodes the protein, is considered homologous to a second protein or nucleic acid molecule if the amino acid or nucleotide sequence of the first protein or nucleic acid molecule is at least about 30% homologous, preferably at least about 50% homologous, and more preferably at least about 65% homologous to the respective sequences of the second protein or nucleic acid molecule. In the case of proteins having high homology, the amino acid or nucleotide sequence of the first protein or nucleic acid molecule is at least about 75% homologous, preferably at least about 85% homologous, and more preferably at least about 95% homologous to the amino acid or nucleotide sequence of the second protein or nucleic acid molecule.

Combinations of mouse oligonucleotide pairs are used as PCR primers to amplify the human homologs from the cells to account for sequence divergence. The remainder of the procedure for obtaining the human flk homologs are similar to those described above for obtaining mouse flk receptors. The less than perfect homology between the human flk homologs and the mouse oligonucleotides is taken into account in determining the stringency of the hybridization conditions.

<u>Assay for expression of Receptors on Stem Cells</u>

In order to demonstrate the expression of flk receptors on the surface of primitive hematopoietic stem cells, antibodies that recognize the receptor are raised. The receptor may be the entire protein as it exists in nature, or an antigenic fragment of the whole protein. Preferably, the fragment comprises the predicted extra-cellular portion of the molecule.

Antigenic fragments may be identified by methods known in the art. Fragments containing antigenic sequences may be selected on the basis of generally accepted criteria of potential antigenicity and/or exposure. Such criteria

include the hydrophilicity and relative antigenic index, as determined by surface exposure analysis of proteins. The determination of appropriate criteria is known to those skilled in the art, and has been described, for example, by Hopp et al, Proc. Nat'l Acad. Sci. USA 78, 3824-3828 (1981); Kyte et al, J. Mol. Biol. 157, 105-132 (1982); Emini, J. Virol. 55, 836-839 (1985); Jameson et al, CA BIOS 4, 181-186 (1988); and Karplus et al, Naturwissenschaften 72, 212-213 (1985). Amino acid domains predicted by these criteria to be surface exposed are selected preferentially over domains predicted to be more hydrophobic or hidden.

The proteins and fragments of the receptors to be used as antigens may be prepared by methods known in the art. Such methods include isolating or synthesizing DNA encoding the proteins and fragments, and using the DNA to produce recombinant proteins, as described above.

Fragments of proteins and DNA encoding the fragments may be chemically synthesized by methods known in the art from individual amino acids and nucleotides. Suitable methods for synthesizing protein fragments are described by Stuart and Young in "Solid Phase Peptide Synthesis," Second Edition, Pierce Chemical Company (1984). Suitable methods for synthesizing DNA fragments are described by Caruthers in Science 230, 281-285 (1985).

If the receptor fragment defines the epitope, but is too short to be antigenic, it may be conjugated to a carrier molecule in order to produce antibodies. Some suitable carrier molecules include keyhole limpet hemocyanin, Ig sequences, TrpE, and human or bovine serum albumen. Conjugation may be carried out by methods known in the art. One such method is to combine a cysteine residue of the fragment with a cysteine residue on the carrier molecule.

The antibodies are preferably monoclonal. Monoclonal antibodies may be produced by methods known in the art. These methods include the immunological method described by

Kohler and Milstein in Nature 256, 495-497 (1975) and Campbell in "Monoclonal Antibody Technology, The Production and Characterization of Rodent and Human Hybridomas" in Burdon et al., Eds, Laboratory Techniques in Biochemistry and Molecular Biology, Volume 13, Elsevier Science Publishers, Amsterdam (1985); as well as by the recombinant DNA method described by Huse et al in Science 246, 1275-1281 (1989).

Polyclonal or monoclonal antisera shown to be reactive with receptor-encoded native proteins, such as with flk-1 and flk-2 encoded proteins, expressed on the surface of viable cells are used to isolate antibody-positive cells. One method for isolating such cells is flow cytometry; see, for example, Loken et al., European patent application 317,156. The cells obtained are assayed for stem cells by engraftment into radiation-ablated hosts by methods known in the art; see, for example, Jordan et al., Cell 61, 953-963 (1990).

Criteria for Novel Stem Cell Receptor Tyrosine Kinases Expressed in Stem Cells

Additional novel receptor tyrosine kinase cDNAs are obtained by amplifying cDNAs from stem cell populations using oligonucleotides as PCR primers; see above. Examples of suitable oligonucleotides are PTK1 and PTK2, which were described by Wilks et al. in Proc. Natl. Acad. Sci. USA 86, 1603-1607 (1989). Novel cDNA is selected on the basis of differential hybridization screening with probes representing known kinases. The cDNA clones hybridizing only at low stringency are selected and sequenced. The presence of the amino acid triplet DFG confirms that the sequence represents a kinase. The diagnostic methionine residue in the WMAPES motif is indicative of a receptor-like kinase, as described above. Potentially novel sequences obtained are compared to available sequences using databases such as Genbank in order to confirm uniqueness. Gene-specific oligonucleotides are prepared as described above based on the sequence obtained. The oligonucleotides are used to analyze stem cell enriched and depleted populations for expression. Such cell populations in mice are described, for example, by Jordan et

al. in Cell 61, 953-956 (1990); Ikuta et al. in Cell 62, 863-864 (1990); Spangrude et al. in Science 241, 58-62 (1988); and Szilvassy et al. in Blood 74, 930-939 (1989). Examples of such human cell populations are described as $CD33^{-}CD34^{+}$ by Andrews et al. in the Journal of Experimental Medicine 169, 1721-1731 (1989). Other human stem cell populations are described, for example, in Civin et al., European Patent Application 395,355 and in Loken et al., European Patent Application 317,156.

Isolating Ligands and Nucleic Acid Molecules Encoding Ligands

Cells that may be used for obtaining ligands include stromal cells, for example stromal cells from fetal liver, fetal spleen, fetal thymus and fetal or adult bone marrow. Cell lines expressing ligands are established and screened.

For example, cells such as stromal (non-hematopoietic) cells from fetal liver are immortalized by known methods. Examples of known methods of immortalizing cells include transduction with a temperature sensitive SV40 T-antigen expressed in a retroviral vector. Infection of fetal liver cells with this virus permits the rapid and efficient establishment of multiple independent cell lines. These lines are screened for ligand activity by methods known in the art, such as those outlined below.

Ligands for the receptors of the invention, such as flk-1 and flk-2, may be obtained from the cells in several ways. For example, a bioassay system for ligand activity employs chimeric tagged receptors; see, for example, Flanagan et al., Cell 63, 185-194 (1990). One strategy measures ligand binding directly via a histochemical assay. Fusion proteins comprising the extracellular receptor domains and secretable alkaline phosphatase (SEAP) are constructed and transfected into suitable cells such as NIH/3T3 or COS cells. Flanagan et al. refer to such DNA or amino acid constructs as APtag followed by the name of the receptor - i.e. APtag-c-kit. The fusion proteins bind with high affinity to cells expressing

surface-bound ligand. Binding is detectable by the enzymatic activity of the alkaline phosphatase secreted into the medium. The bound cells, which are often stromal cells, are isolated from the APtag-receptor complex.

For example, some stromal cells that bind APtag-flk1 and APtag-flk2 fusion proteins include mouse fetal liver cells (see example 1); human fetal spleen cells (see example 3); and human fetal liver (example 3). Some stromal fetal thymus cells contain flk-1 ligand (example 3).

To clone the cDNA that encodes the ligand, a cDNA library is constructed from the isolated stromal cells in a suitable expression vector, preferably a phage such as CDM8, pSV Sport (BRL Gibco) or piH3, (Seed et al., Proc. Natl. Acad. Sci. USA 84, 3365-3369 (1987)). The library is transfected into suitable host cells, such as COS cells. Cells containing ligands on their surface are detected by known methods, see above.

In one such method, transfected COS cells are distributed into single cell suspensions and incubated with the secreted alkaline phosphatase-flk receptor fusion protein, which is present in the medium from NIH/3T3 or COS cells prepared by the method described by Flanagan et al., see above. Alkaline phosphatase-receptor fusion proteins that are not bound to the cells are removed by centrifugation, and the cells are panned on plates coated with antibodies to alkaline phosphatase. Bound cells are isolated following several washes with a suitable wash reagent, such as 5% fetal bovine serum in PBS, and the DNA is extracted from the cells. Additional details of the panning method described above may be found in an article by Seed et al., Proc. Natl. Acad. Sci. USA 84, 3365-3369 (1987).

In a second strategy, the putative extracellular ligand binding domains of the receptors are fused to the transmembrane and kinase domains of the human c-fms tyrosine kinase and introduced into 3T3 fibroblasts. The human c-fms

kinase is necessary and sufficient to transduce proliferative signals in these cells after appropriate activation i.e. with the flk-1 or flk-2 ligand. The 3T3 cells expressing the chimeras are used to screen putative sources of ligand in a cell proliferation assay.

An alternate approach for isolating ligands using the fusion receptor-expressing 3T3 cells and insertional activation is also possible. A retrovirus is introduced into random chromosomal positions in a large population of these cells. In a small fraction, the retrovirus is inserted in the vicinity of the ligand-encoding gene, thereby activating it. These cells proliferate due to autocrine stimulation of the receptor. The ligand gene is "tagged" by the retrovirus, thus facilitating its isolation.

Examples

Example 1. Cells containing mouse flk-1 and flk-2 ligands. Murine stromal cell line 2018.

In order to establish stromal cell lines, fetal liver cells are disaggregated with collagen and grown in a mixture of Dulbecco's Modified Eagle's Medium (DMEM) and 10% heat-inactivated fetal calf serum at 37°C. The cells are immortalized by standard methods. A suitable method involves introducing DNA encoding a growth regulating- or oncogene-encoding sequence into the target host cell. The DNA may be introduced by means of transduction in a recombinant viral particle or transfection in a plasmid. See, for example, Hammerschmidt et al., Nature 340, 393-397 (1989) and Abcouwer et al, Biotechnology 7, 939-946 (1989). Retroviruses are the preferred viral vectors, although SV40 and Epstein-Barr virus can also serve as donors of the growth-enhancing sequences. A suitable retrovirus is the ecotropic retrovirus containing a temperature sensitive SV40 T-antigen (tsA58) and a G418 resistance gene described by McKay in Cell 66, 713-729 (1991). After several days at 37°C, the temperature of the medium is lowered to 32°C. Cells are selected with G418 (0.5

mg/ml). The selected cells are expanded and maintained.

A mouse stromal cell line produced by this procedure is called 2018 and was deposited on October 30, 1991 in the American Type Culture Collection, Rockville, Maryland, USA (ATCC); accession number CRL 10907.

Example 2. Cells containing human flk-1 and flk-2 ligands.

Human fetal liver (18, 20, and 33 weeks after abortion), spleen (18 weeks after abortion), or thymus (20 weeks after abortion) is removed at the time of abortion and stored on ice in a balanced salt solution. After mincing into 1 mm fragments and forcing through a wire mesh, the tissue is washed one time in Hanks Balanced Salt Solution (HBSS).

The disrupted tissue is centrifuged at 200 xg for 15 minutes at room temperature. The resulting pellet is resuspended in 10-20 ml of a tissue culture grade trypsin-EDTA solution (Flow Laboratories). The resuspended tissue is transferred to a sterile flask and stirred with a stirring bar at room temperature for 10 minutes. One ml of heat-inactivated fetal bovine calf serum (Hyclone) is added to a final concentration of 10% in order to inhibit trypsin activity. Collagenase type IV (Sigma) is added from a stock solution (10 mg/ml in HBSS) to a final concentration of 100 μg/ml in order to disrupt the stromal cells. The tissue is stirred at room temperature for an additional 2.5 hours; collected by centrifugation (400xg, 15 minutes); and resuspended in "stromal medium," which contains Iscove's modification of DMEM supplemented with 10% heat-inactivated fetal calf serum, 5% heat-inactivated human serum (Sigma), 4 mM L-glutamine, 1x sodium pyruvate, (stock of 100x Sigma), 1x non-essential amino acids (stock of 100x, Flow), and a mixture of antibiotics kanomycin, neomycin, penicillin, streptomycin. Prior to resuspending the pellet in the stromal medium, the pellet is washed one time with HBSS. It is convenient to suspend the cells in 60 ml of medium. The number of cultures depends on the amount of

tissue.

Example 3. Isolating Stromal cells

Resuspended Cells (example 2) that are incubated at 37°C with 5% carbon dioxide begin to adhere to the plastic plate within 10-48 hours. Confluent monolayers may be observed within 7-10 days, depending upon the number of cells plated in the initial innoculum. Non-adherent and highly refractile cells adhering to the stromal cell layer as colonies are separately removed by pipetting and frozen. Non-adherent cells are likely sources of populations of self-renewing stem cells containing flk-2. The adherent stromal cell layers are frozen in aliquots for future studies or expanded for growth in culture.

An unexpectedly high level of APtag-flk-2 fusion protein binding to the fetal spleen cells is observed. Two fetal spleen lines are grown in "stromal medium," which is described in example 2.

Non-adherent fetal stem cells attach to the stromal cells and form colonies (colony forming unit - CFU). Stromal cells and CFU are isolated by means of sterile glass cylinders and expanded in culture. A clone, called Fsp 62891, contains the flk-2 ligand. Fsp 62891 was deposited in the American Type Culture Collection, Rockville, Maryland, U.S.A on November 21, 1991, accession number CRL 10935.

Fetal liver and fetal thymus cells are prepared in a similar way. Both of these cell types produce ligands of flk-1 and, in the case of liver, some flk-2. One such fetal thymus cell line, called F.thy 62891, and one such fetal liver cell line, called FL 62891, were deposited in the American Type Culture Collection, Rockville, Maryland, U.S.A on November 21, 1991 and April 2, 1992, respectively, accession numbers CRL 10936 and CRL 11005, respectively.

Stable human cell lines are prepared from fetal cells

with the same temperature sensitive immortalizing virus used to prepare the murine cell line described in example 1.

Example 4. Isolation of human stromal cell clone

Highly refractile cells overgrow patches of stromal cells, presumably because the stromal cells produce factors that allow the formation of the CFU. To isolate stromal cell clones, sterile glass cylinders coated with vacuum grease are positioned over the CFU. A trypsin-EDTA solution (100 ml) is added in order to detach the cells. The cells are added to 5 ml of stromal medium and each (clone) plated in a single well of 6-well plate.

Example 5. Plasmid (AP-tag) for expressing secretable alkaline phosphatase (SEAP)

Plasmids that express secretable alkaline phosphatase are described by Flanagan and Leder in Cell 63, 185-194 (1990). The plasmids contain a promoter, such as the LTR promoter; a polylinker, including HindIII and BglII; DNA encoding SEAP; a poly-A signal; and ampicillin resistance gene; and replication site.

Example 6. Plasmid for expressing APtag-flk-2 and APtag-flk-1 fusion proteins

Plasmids that express fusion proteins of SEAP and the extracellular portion of either flk-1 or flk-2 are prepared in accordance with the protocols of Flanagan and Leder in Cell 63, 185-194 (1990) and Berger et al., Gene 66, 1-10 (1988). Briefly, a HindIII-Bam HI fragment containing the extracellular portion of flk-1 or flk-2 is prepared and inserted into the HindIII-BglII site of the plasmid described in example 5.

Example 7. Production Of APtag-flk-1 Or -flk-2 Fusion Protein

The plasmids from Example 6 are transfected into Cos-7 cells by DEAE-dextran (as described in Current Protocols in Molecular Biology, Unit 16.13, "Transient Expression of Proteins Using Cos Cells," 1991); and cotransfected with a selectable marker, such as pSV7neo, into NIH/3T3 cells by calcium precipitation. The NIH/3T3 cells are selected with 600μg/ml G418 in 100 mm plates. Over 300 clones are screened for secretion of placental alkaline phosphatase activity. The assay is performed by heating a portion of the supernatant at 65°C for 10 minutes to inactivate background phosphatase activity, and measuring the OD_{405} after incubating with 1M diethanolamine (pH 9.8), 0.5 mM $MgCl_2$, 10 mM L-homoarginine (a phosphatase inhibitor), 0.5 mg/ml BSA, and 12 mM p-nitrophenyl phosphate. Human placental alkaline phosphatase is used to perform a standard curve. The APtaq-flk-1 clones (F-1AP21-4) produce up to 10 μg alkaline phosphatase activity/ml and the APtaq-flk-2 clones (F-2AP26-0) produce up to 0.5 μg alkaline phosphatase activity/ml.

Example 8. Assay For APtaq-flk-1 Or APtaq-flk-2 Binding To Cells

The binding of APtaq-flk-1 or APtag-flk-2 to cells containing the appropriate ligand is assayed by standard methods. See, for example, Flanagan and Leder, Cell 63:185-194, 1990). Cells (i.e., mouse stromal cells, human fetal liver, spleen or thymus, or various control cells) are grown to confluency in six-well plates and washed with HBHA (Hank's balanced salt solution with 0.5 mg/ml BSA, 0.02% NaN_3, 20 mM HEPES, pH 7.0). Supernatants from transfected COS or NIH/3T3 cells containing either APtaq-flk-1 fusion protein, APtag-flk-2 fusion protein, or APtag without a receptor (as a control) are added to the cell monolayers and incubated for two hours at room temperature on a rotating platform. The concentration of the APtaq-flk-1 fusion protein, APtag-flk-2 fusion protein, or APtag without a receptor is 60 ng/ml of alkaline phosphatase as determined by the standard alkaline

phosphatase curve (see above). The cells are then rinsed seven times with HBHA and lysed in 350 µl of 1% Triton X-100, 10 mM Tris-HCl (pH 8.0). The lysates are transferred to a microfuge tube, along with a further 150 µl rinse with the same solution. After vortexing vigorously, the samples are centrifuged for five minutes in a microfuge, heated at 65°C for 12 minutes to inactivate cellular phosphatases, and assayed for phosphatase activity as described previously. Results of experiments designed to show the time and dose responses of binding between stromal cells containing the ligands to flk-2 and flk-1 (2018) and APtag-flk-2, APtag-flk-1 and APtag without receptor (as a control) are shown in Figures 3 and 4, respectively.

Example 8A. Plasmids for expressing flk1/fms and flk2/fms fusion proteins

Plasmids that express fusion proteins of the extracellular portion of either flk-1 or flk-2 and the intracellular portion of c-fms (also known as colony-stimulating factor-1 receptor) are prepared in a manner similar to that described under Example 6 (Plasmid for expressing APtag-flk-2 and APtag-flk-1 fusion proteins). Briefly, a Hind III - Bam HI fragment containing the extracellular portion of flk1 or flk2 is prepared and inserted into the Hind III - Bgl II site of a pLH expression vector containing the intracellular portion of c-fms.

8B. Expression of flk1/fms or flk2/fms in 3T3 cells

The plasmids from Example 11 are transfected into NIH/3T3 cells by calcium. The intracellular portion of c-fms is detected by Western blotting.

Example 9. Cloning and Expression of cDNA Coding For Mouse Ligand To flk-1 and flk-2 Receptors

cDNA expressing mouse ligand for flk-1 and flk-2 is

prepared by known methods. See, for example, Seed, B., and Aruffo, A. PNAS 84:3365-3369, 1987; Simmons, D. and Seed, B. J. Immunol. 141:2797-2800; and D'Andrea, A.D., Lodish, H.F. and Wong, G.G. Cell 57:277-285, 1989).

The protocols are listed below in sequence: (a) RNA isolation; (b) poly A RNA preparation; (c) cDNA synthesis; (d) cDNA size fractionation; (e) propagation of plasmids (vector); (f) isolation of plasmid DNA; (g) preparation of vector pSV Sport (BRL Gibco) for cloning; (h) compilation of buffers for the above steps; (i) Transfection of cDNA encoding Ligands in Cos 7 Cells; (j) panning procedure; (k) Expression cloning of flk-1 or flk-2 ligand by establishment of an autocrine loop.

9a. Guanidinium thiocyanate/LiCl Protocol for RNA Isolation

For each ml of mix desired, 0.5 g guanidine thiocyanate (GuSCN) is dissolved in 0.55 ml of 25% LiCl (stock filtered through 0.45 micron filter). 20 μl of mercaptoethanol is added. (The resulting solution is not good for more than about a week at room temperature.)

The 2018 stromal cells are centrifuged, and 1 ml of the solution described above is added to up to 5 x 10^7 cells. The cells are sheared by means of a polytron until the mixture is non-viscous. For small scale preparations (<10^8 cells), the sheared mixture is layered on 1.5 ml of 5.7M CsCl (RNase free; 1.26 g CsCl added to every ml 10 mM EDTA pH8), and overlaid with RNase-free water if needed. The mixture is spun in an SW55 rotor at 50 krpm for 2 hours. For large scale preparations, 25 ml of the mixture is layered on 12 ml CsCl in an SW28 tube, overlaid as above, and spun at 24 krpm for 8 hours. The contents of the tube are aspirated carefully with a sterile pasteur pipet connected to a vacuum flask. Once past the CsCl interface, a band around the tube is scratched with the pipet tip to prevent creeping of the layer on the wall down the tube. The remaining CsCl solution is aspirated. The resulting pellet is taken up in

water, but not redissolved. 1/10 volume of sodium acetate and three volumes of ethanol are added to the mixture, and spun. The pellet is resuspended in water at 70°C, if necessary. The concentration of the RNA is adjusted to 1 mg/ml and frozen.

It should be noted that small RNA molecules (e.g., 5S) do not come down. For small amounts of cells, the volumes are scaled down, and the mixture is overlaid with GuSCN in RNase-free water on a gradient (precipitation is inefficient when RNA is dilute).

<u>9b. Poly A⁻ RNA preparation</u>

(All buffers mentioned are compiled separately below)

A disposable polypropylene column is prepared by washing with 5M NaOH and then rinsing with RNase-free water. For each milligram of total RNA, approximately 0.3 ml (final packed bed) of oligo dT cellulose is added. The oligo dT cellulose is prepared by resuspending approximately 0.5 ml of dry powder in 1 ml of 0.1M NaOH and transferring it into the column, or by percolating 0.1M NaOH through a previously used column. The column is washed with several column volumes of RNase-free water until the pH is neutral, and rinsed with 2-3 ml of loading buffer. The column bed is transferred to a sterile 15 ml tube using 4-6 ml of loading buffer.

Total RNA from the 2018 cell line is heated to 70°C for 2-3 minutes. LiCl from RNase-free stock is added to the mixture to a final concentration of 0.5M. The mixture is combined with oligo dT cellulose in the 15 ml tube, which is vortexed or agitated for 10 minutes. The mixture is poured into the column, and washed with 3 ml loading buffer, and then with 3 ml of middle wash buffer. The mRNA is eluted directly into an SW55 tube with 1.5 ml of 2 mM EDTA and 0.1% SDS, discarding the first two or three drops.

The eluted mRNA is precipitated by adding 1/10 volume of

3M sodium acetate and filling the tube with ethanol. The contents of the tube are mixed, chilled for 30 minutes at -20°C, and spun at 50 krpm at 5°C for 30 minutes. After the ethanol is decanted, and the tube air dried, the mRNA pellet is resuspended in 50-100 μl of RNase-free water. 5 μl of the resuspended mRNA is heated to 70°C in MOPS/EDTA/formaldehyde, and examined on an RNase-free 1% agarose gel.

9c. cDNA Synthesis

The protocol used is a variation of the method described by Gubler and Hoffman in Gene 25, 263-270 (1983).

1. First Strand. 4 μg of mRNA is added to a microfuge tube, heated to approximately 100°C for 30 seconds, quenched on ice. The volume is adjusted to 70μl with RNAse-free water. 20 μl of RT1 buffer, 2 μl of RNAse inhibitor (Boehringer 36 u/μl), 1 μl of 5 μg/μl of oligo dT (Collaborative Research), 2.5 μl of 20 mM dXTP's (ultrapure - US Biochemicals), 1 μl of 1M DTT and 4 μl of RT-XL (Life Sciences, 24 u/μl) are added. The mixture is incubated at 42°C for 40 minutes, and inactivated by heating at 70°C for 10 minutes.

2. Second Strand. 320 μl of RNAse-free water, 80 μl of RT2 buffer, 5 μl of DNA Polymerase I (Boehringer, 5 U/μl), 2 μl RNAse H (BRL 2 u/μl) are added to the solution containing the first strand. The solution is incubated at 15°C for one hour and at 22°C for an additional hour. After adding 20 μl of 0.5M EDTA, pH 8.0, the solution is extracted with phenol and precipitated by adding NaCl to 0.5M linear polyacrylamide (carrier) to 20 μg/ml, and filling the tube with EtOH. The tube is spun for 2-3 minutes in a microfuge, vortexed to dislodge precipitated material from the wall of the tube, and respun for one minute.

3. Adaptors. Adaptors provide specific restriction sites to facilitate cloning, and are available from BRL

Gibco, New England Biolabs, etc. Crude adaptors are resuspended at a concentration of 1 $\mu g/\mu l$. $MgSO_4$ is added to a final concentration of 10 mM, followed by five volumes of EtOH. The resulting precipitate is rinsed with 70% EtOH and resuspended in TE at a concentration of 1 $\mu g/\mu l$. To kinase, 25 μl of resuspended adaptors is added to 3 μl of 10X kinasing buffer and 20 units of kinase. The mixture is incubated at 37°C overnight. The precipitated cDNA is resuspended in 240 μl of TE (10/1). After adding 30 μl of 10X low salt buffer, 30 μl of 10X ligation buffer with 0.1mM ATP, 3 μl (2.4 μg) of kinased 12-mer adaptor sequence, 2 μl (1.6 μg) of kinased 8-mer adaptor sequence, and 1 μl of T4 DNA ligase (BioLabs, 400 u/μl, or Boehringer, 1 Weiss unit ml), the mixture is incubated at 15°C overnight. The cDNA is extracted with phenol and precipitated as above, except that the extra carrier is omitted, and resuspended in 100 μl of TE.

9d. cDNA Size Fractionation.

A 20% KOAc, 2 mM EDTA, 1 $\mu g/ml$ ethidium bromide solution and a 5% KOAc, 2 mM EDTA, 1 $\mu g/ml$ ethidium bromide solution are prepared. 2.6 ml of the 20% KOAc solution is added to the back chamber of a small gradient maker. Air bubbles are removed from the tube connecting the two chambers by allowing the 20% solution to flow into the front chamber and forcing the solution to return to the back chamber by tilting the gradient maker. The passage between the chambers is closed, and 2.5 ml of 5% solution is added to the front chamber. Any liquid in the tubing from a previous run is removed by allowing the 5% solution to flow to the end of the tubing, and then to return to its chamber. The apparatus is placed on a stirplate, and, with rapid stirring, the topcock connecting the two chambers and the front stopcock are opened. A polyallomer 5W55 tube is filled from the bottom with the KOAc solution. The gradient is overlaid with 100 μl of cDNA solution, and spun for three hours at 50k rpm at 22°C. To collect fractions from the gradient, the SW55 tube is pierced close to the bottom of the tube with a butterfly

infusion set (with the luer hub clipped off). Three 0.5 ml fractions and then six 0.25 ml fractions are collected in microfuge tubes (approximately 22 and 11 drops, respectively). The fractions are precipitated by adding linear polyacrylamide to 20 μg/ml and filling the tube to the top with ethanol. The tubes are cooled, spun in a microfuge tube for three minutes, vortexed, and respun for one minute. The resulting pellets are rinsed with 70% ethanol and respun, taking care not to permit the pellets to dry to completion. Each 0.25 ml fraction is resuspended in 10 μl of TE, and 1 μl is run on a 1% agarose minigel. The first three fractions, and the last six which contain no material smaller than 1 kb are pooled.

9e. Propagation of Plasmids

SupF plasmids are selected in nonsuppressing bacterial hosts containing a second plasmid, p3, which contains amber mutated ampicillin and tetracycline drug resistance elements. See Seed, Nucleic Acids Res., 11, 2427-2445 (1983). The p3 plasmid is derived from RP1, is 57 kb in length, and is a stably maintained, single copy episome. The ampicillin resistance of this plasmid reverts at a high rate so that amp^r plasmids usually cannot be used in p3-containing strains. Selection for tetracycline resistance alone is almost as good as selection for ampicillin-tetracycline resistance. However, spontaneous appearance of chromosomal suppressor tRNA mutations presents an unavoidable background (frequency about 10^{-9}) in this system. Colonies arising from spontaneous suppressor mutations are usually larger than colonies arising from plasmid transformation. Suppressor plasmids are selected in Luria broth (LB) medium containing ampicillin at 12.5 μg/ml and tetracycline at 7.5 μg/ml. For scaled-up plasmid preparations, M9 Casamino acids medium containing glycerol (0.8%) is employed as a carbon source. The bacteria are grown to saturation.

Alternatively, pSV Sport (BRL, Gaithersberg, Maryland) may be employed to provide SV40 derived sequences for

replication, transcription initiation and termination in COS 7 cells, as well as those sequences necessary for replication and ampicillin resistance in E. coli.

9f. Isolation of Vector DNA/Plasmid

One liter of saturated bacterial cells are spun down in J6 bottles at 4.2k rpm for 25 minutes. The cells are resuspended in 40 ml 10 mM EDTA, pH 8. 80 ml 0.2M NaOH and 1% SDS are added, and the mixture is swirled until it is clear and viscous. 40 ml 5M KOAc, pH 4.7 (2.5M KOAc, 2.5M HOAc) is added, and the mixture is shaken semi-vigorously until the lumps are approximately 2-3 mm in size. The bottle is spun at 4.2k rpm for 5 minutes. The supernatant is poured through cheesecloth into a 250 ml bottle, which is then filled with isopropyl alcohol and centrifuged at 4.2k rpm for 5 minutes. The bottle is gently drained and rinsed with 70% ethanol, taking care not to fragment the pellet. After inverting the bottle and removing traces of ethanol, the mixture is resuspended in 3.5 ml Tris base/EDTA (20 mM/10 mM). 3.75 ml of resuspended pellet and 0.75 ml 10 mg/ml ethidium bromide are added to 4.5 g CsCl. VTi80 tubes are filled with solution, and centrifuged for at least 2.5 hours at 80k rpm. Bands are extracted by visible light with 1 ml syringe and 20 gauge or lower needle. The top of the tube is cut off with scissors, and the needle is inserted upwards into the tube at an angle of about 30 degrees with respect to the tube at a position about 3 mm beneath the band, with the bevel of the needle up. After the band is removed, the contents of the tube are poured into bleach. The extracted band is deposited in a 13 ml Sarstedt tube, which is then filled to the top with n-butanol saturated with 1M NaCl extract. If the amount of DNA is large, the extraction procedure may be repeated. After aspirating the butanol into a trap containing 5M NaOH to destroy ethidium, an approximately equal volume of 1M ammonium acetate and approximately two volumes of 95% ethanol are added to the DNA, which is then spun at 10k rpm for 5 minutes. The pellet is rinsed carefully with 70% ethanol, and dried with a swab

or lyophilizer.

9g. Preparation of Vector for Cloning

20 µg of vector is cut in a 200 µl reaction with 100 units of BstXI (New York Biolabs) at 50°C overnight in a well thermostated, circulating water bath. Potassium acetate solutions (5 and 20%) are prepared in 5W55 tubes as described above. 100 µl of the digested vector is added to each tube and spun for three hours, 50k rpm at 22°C. Under 300 nm UV light, the desired band is observed to migrate 2/3 of the length of the tube. Forward trailing of the band indicates that the gradient is overloaded. The band is removed with a 1 ml syringe fitted with a 20 gauge needle. After adding linear polyacrylamide and precipitating the plasmid by adding three volumes of ethanol, the plasmid is resuspended in 50 µl of TE. Trial ligations are carried out with a constant amount of vector and increasing amounts of cDNA. Large scale ligation are carried out on the basis of these trial ligations. Usually the entire cDNA prep requires 1-2 µg of cut vector.

9h. Buffers

Loading Buffer:	.5M LiCl, 10 mM Tris pH 7.5, 1 mM EDTA .1% SDS.
Middle Wash Buffer:	.15M LiCl, 10 mM Tris pH 7.5, 1 mM EDTA .1% SDS.
RT1 Buffer:	.25M Tris pH 8.8 (8.2 at 42°), .25M KCl, 30 mM $MgCl_2$.
RT2 Buffer:	.1M Tris pH 7.5, 25 mM MgCl2, .5M KCl, .25 mg/ml BSA, 50 mM dithiothreitol (DTT).
10X Low Salt:	60 mM Tris pH 7.5, 60 mM $MgCl_2$, 50 mM NaCl, 2.5 mg/ml BSA 70 mM DME
10X Ligation Additions:	1 mM ATP, 20 mM DTT, 1 mg/ml BSA 10 mM spermidine.
10X Kinasing Buffer:	.5M Tris pH 7.5, 10 mM ATP, 20 mM DTT, 10 mM spermidine, 1 mg/ml BSA

100 mM $MgCl_2$

9i. Transfection of cDNA encoding Ligands in Cos 7 Cells

Cos 7 cells are split 1:5 into 100 mm plates in Dulbecco's modified Eagles medium (DME)/10% fetal calf serum (FCS), and allowed to grow overnight. 3 ml Tris/DME (0.039M Tris, pH 7.4 in DME) containing 400 µg/ml DEAE-dextran (Sigma, D-9885) is prepared for each 100 mm plate of Cos 7 cells to be transfected. 10 µg of plasmid DNA preparation per plate is added. The medium is removed from the Cos-7 cells and the DNA/DEAE-dextran mixture is added. The cells are incubated for 4.5 hours. The medium is removed from the cells, and replaced with 3 ml of DME containing 2% fetal calf serum (FCS) and 0.1 mM chloroquine. The cells are incubated for one hour. After removing the chloroquine and replacing with 1.5 ml 20% glycerol in PBS, the cells are allowed to stand at room temperature for one minute. 3 ml Tris/DME is added, and the mixture is aspirated and washed two times with Tris/DME. 10 ml DME/10% FCS is added and the mixture is incubated overnight. The transfected Cos 7 cells are split 1:2 into fresh 100 mm plates with (DME)/10% FCS and allowed to grow.

9j. Panning Procedure for Cos 7 cells Expressing Ligand

1) Antibody-coated plates:

Bacteriological 100 mm plates are coated for 1.5 hours with rabbit anti-human placental alkaline phosphatase (Dako, California) diluted 1:500 in 10 ml of 50 mM Tris.HCl, pH 9.5. The plates are washed three times with 0.15M NaCl, and incubated with 3 mg BSA/ml PBS overnight. The blocking solution is aspirated, and the plates are utilized immediately or frozen for later use.

2) Panning cells:

The medium from transfected Cos 7 cells is aspirated,

and 3 ml PBS/0.5 mM EDTA/0.02% sodium azide is added. The plates are incubated at 37°C for thirty minutes in order to detach the cells. The cells are triturated vigorously with a pasteur pipet and collected in a 15 ml centrifuge tube. The plate is washed with a further 2 ml PBS/EDTA/azide solution, which is then added to the centrifuge tube. After centrifuging at 200 xg for five minutes, the cells are resuspended in 3 ml of APtaq-flk-1 (F-1AP21-4) or flk-2 (F-2AP26-0) supernatant from transfected NIH/3T3 cells (see Example 7.), and incubated for 1.5 hours on ice. The cells are centrifuged again at 200 xg for five minutes. The supernatant is aspirated, and the cells are resuspended in 3 ml PBS/EDTA/azide solution. The cell suspension is layered carefully on 3 ml PBS/EDTA/azide/2% Ficoll, and centrifuged at 200 xg for four minutes. The supernatant is aspirated, and the cells are resuspended in 0.5 ml PBS/EDTA/azide solution. The cells are added to the antibody-coated plates containing 4 ml PBS/EDTA/azide/5% FBS, and allowed to stand at room temperature one to three hours. Non-adhering cells are removed by washing gently two or three times with 3 ml PBS/5% FBS.

3) <u>Hirt Supernatant:</u>

0.4 ml 0.6% SDS and 10 mM EDTA are added to the panned plates, which are allowed to stand 20 minutes. The viscuous mixture is added by means of a pipet into a microfuge tube. 0.1 ml 5M NaCl is added to the tube, mixed, and chilled on ice for at least five hours. The tube is spun for four minutes, and the supernatant is removed carefully. The contents of the tube are extracted with phenol once, or, if the first interface is not clean, twice. Ten micrograms of linear polyacrylamide (or other carrier) is added, and the tube is filled to the top with ethanol. The resulting precipitate is resuspended in 0.1 ml water or TE. After adding 3 volumes of EtOH/NaOAc, the cells are reprecipitated and resuspended in 0.1 ml water or TE. The cDNA obtained is transfected into any suitable <u>E. coli</u> host by electroporation. Suitable hosts are described in various

catalogs, and include MC1061/p3 or Electromax DH10B Cells of BRL Gibco. The cDNA is extracted by conventional methods.

The above panning procedure is repeated until a pure E. coli clone bearing the cDNA as a unique plasmid recombinant capable of transfecting mammalian cells and yielding a positive panning assay is isolated. Normally, three repetitions are sufficient.

9k. Expression cloning of flk1 or flk2 ligand by establishment of an autocrine loop

Cells expressing flk1/fms or flk2/fms (Example 10) are transfected with 20-30 μg of a cDNA library from either flk1 ligand or flk2 ligand expressing stromal cells, respectively. The cDNA library is prepared as described above (a-h). The cells are co-transfected with 1 μg pLTR neo cDNA. Following transfection the cells are passaged 1:2 and cultured in 800 μg/ml of G418 in Dulbecco's medium (DME) supplemented with 10% CS. Approximately 12 days later the colonies of cells are passaged and plated onto dishes coated with poly -D-lysine (1 mg/ml) and human fibronectin (15 μg/ml). The culture medium is defined serum-free medium which is a mixture (3:1) of DME and Ham's F12 medium. The medium supplements are 8 mM $NaHCO_3$, 15 mM HEPES pH 7.4, 3 mM histidine, 4 μM $MnCl_2$, 10 μM ethanolamine, 0.1 μM selenous acid, 2 μM hydrocortisone, 5 μg/ml transferrin, 500 μg/ml bovine serum albumin/linoleic acid complex, and 20 μg/ml insulin (Ref. Zhan, X, et al. Oncogene 1: 369-376,1987). The cultures are refed the next day and every 3 days until the only cells capable of growing under the defined medium condition remain. The remaining colonies of cells are expanded and tested for the presence of the ligand by assaying for binding of APtag - flk1 or APtag - flk2 to the cells (as described in Example 8). The DNA would be rescued from cells demonstrating the presence of the flk1 or flk2 ligand and the sequence.

Example 10. Expression of Ligand cDNA

The cDNA is sequenced, and expressed in a suitable host cell, such as a mammalian cell, preferably COS, CHO or NIH/3T3 cells. The presence of the ligand is confirmed by demonstrating binding of the ligand to APtag-flk2 fusion protein (see above).

Example 11. Chemical Cross Linking of Receptor and Ligand

Cross linking experiments are performed on intact cells using a modification of the procedure described by Blume-Jensen et al et al., EMBO J., 10, 4121-4128 (1991). Cells are cultured in 100mm tissue culture plates to subconfluence and washed once with PBS-0.1% BSA.

To examine chemical cross linking of soluble receptor to membrane-bound ligand, stromal cells from the 2018 stromal cell line are incubated with conditioned media (CM) from transfected 3T3 cells expressing the soluble receptor Flk2-APtag. Cross linking studies of soluble ligand to membrane bound receptor are performed by incubating conditioned media from 2018 cells with transfected 3T3 cells expressing a Flk2-fms fusion construct.

Binding is carried out for 2 hours either at room temperature with CM containing 0.02% sodium azide to prevent receptor internalization or at 4°C with CM (and buffers) supplemented with sodium vanadate to prevent receptor dephosphorylation. Cells are washed twice with PBS-0.1% BSA and four times with PBS.

Cross linking is performed in PBS containing 250 mM disuccinimidyl suberate (DSS; Pierce) for 30 minutes at room temperature. The reaction is quenched with Tris-HCL pH7.4 to a final concentration of 50 mM.

Cells are solubilized in solubilization buffer: 0.5% Triton - X100, 0.5% deoxycholic acid, 20 mM Tris pH 7.4, 150

mM NaCl, 10mM EDTA, 1mM PMFS, 50 mg/ml aprotinin, 2 mg/ml bestatin, 2 mg/ml pepstatin and 10mg/ml leupeptin. Lysed cells are immediately transferred to 1.5 ml Nalgene tubes and solubilized by rolling end to end for 45 minutes at 4°C. Lysates are then centrifuged in a microfuge at 14,000g for 10 minutes. Solubilized cross linked receptor complexes are then retrieved from lysates by incubating supernatants with 10% (v/v) wheat germ lectin-Sepharose 6MB beads (Pharmacia) at 4°C for 2 hours or overnight.

Beads are washed once with Tris-buffered saline (TBS) and resuspended in 2X SDS-polyacrylamide nonreducing sample buffer. Bound complexes are eluted from the beads by heating at 95°C for 5 minutes. Samples are analyzed on 4-12% gradient gels (NOVEX) under nonreducing and reducing conditions (0.35 M 2-mercaptoethanol) and then transferred to PVDF membranes for 2 hours using a Novex blotting apparatus. Blots are blocked in TBS-3% BSA for 1 hour at room temperature followed by incubation with appropriate antibody.

Cross linked Flk2-APtag and Flk2-fms receptors are detected using rabbit polyclonal antibodies raised against human alkaline phosphatase and fms protein, respectively. The remainder of the procedure is carried out according to the instructions provided in the ABC Kit (Pierce). The kit is based on the use of a biotinylated secondary antibody and avidin-biotinylated horseradish peroxidase complex for detection.

SUPPLEMENTAL ENABLEMENT

The invention as claimed is enabled in accordance with the above specification and readily available references and starting materials. Nevertheless, Applicants have deposited with the American Type Culture Collection, Rockville, Md., USA (ATCC) the cell lines listed below:

2018, ATCC accession no. CRL 10907, deposited

October 30, 1991.

Fsp 62891, ATCC accession no. CRL 10935, deposited November 21, 1991.

F.thy 62891, ATCC accession no. CRL 10936, deposited November 21, 1991.

FL 62891, ATCC accession no. CRL 11005, deposited April 2, 1992.

These deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and the regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture for 30 years from date of deposit. The organisms will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Applicants and ATCC which assures unrestricted availability upon issuance of the pertinent U.S. patent. Availability of the deposited strains is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: TRUSTEES OF PRINCETON UNIVERSITY

(ii) TITLE OF INVENTION: Totipotent Hematopoietic Stem Cell Receptors And Their Ligands

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: IMCLONE SYSTEMS INCORPORATED
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(D) STATE: NEW YORK
(E) COUNTRY: US
(F) ZIP: 10014

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 02-APR-1992
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: FEIT, IRVING N.

(B) REGISTRATION NUMBER: 28,601
(C) REFERENCE/DOCKET NUMBER: LEM-3-PPPPT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 212-645-1405
(B) TELEFAX: 212-645-2054

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3453 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 31..3009

(ix) FEATURE:
(A) NAME/KEY: mat_peptide

(B) LOCATION: 31..3006

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GCGGCCTGGC TACCGCGCGC TCCGGAGGCC ATG CGG GCG TTG GCG CAG CGC AGC
                                 Met Arg Ala Leu Ala Gln Arg Ser
                                   1               5

GAC CGG CGG CTG CTG CTG CTT GTT GTT TTG TCA GTA ATG ATT CTT GAG
Asp Arg Arg Leu Leu Leu Leu Val Val Leu Ser Val Met Ile Leu Glu
     10                  15                  20

ACC GTT ACA AAC CAA GAC CTG CCT GTG ATC AAG TGT GTT TTA ATC AGT
Thr Val Thr Asn Gln Asp Leu Pro Val Ile Lys Cys Val Leu Ile Ser
 25                  30                  35                  40

CAT GAG AAC AAT GGC TCA TCA GCG GGA AAG CCA TCA TCG TAC CGA ATG
His Glu Asn Asn Gly Ser Ser Ala Gly Lys Pro Ser Ser Tyr Arg Met
                 45                  50                  55

GTG CGA GGA TCC CCA GAA GAC CTC CAG TGT ACC CCG AGG CGC CAG AGT
Val Arg Gly Ser Pro Glu Asp Leu Gln Cys Thr Pro Arg Arg Gln Ser
             60                  65                  70

GAA GGG ACG GTA TAT GAA GCG GCC ACC GTG GAG GTG GCC GAG TCT GGG
Glu Gly Thr Val Tyr Glu Ala Ala Thr Val Glu Val Ala Glu Ser Gly
         75                  80                  85

TCC ATC ACC CTG CAA GTG CAG CTC GCC ACC CCA GGG GAC CTT TCC TGC
Ser Ile Thr Leu Gln Val Gln Leu Ala Thr Pro Gly Asp Leu Ser Cys
     90                  95                 100

CTC TGG GTC TTT AAG CAC AGC TCC CTG GGC TGC CAG CCG CAC TTT GAT
Leu Trp Val Phe Lys His Ser Ser Leu Gly Cys Gln Pro His Phe Asp
105                 110                 115                 120

TTA CAA AAC AGA GGA ATC GTT TCC ATG GCC ATC TTG AAC GTG ACA GAG
Leu Gln Asn Arg Gly Ile Val Ser Met Ala Ile Leu Asn Val Thr Glu
                125                 130                 135

ACC CAG GCA GGA GAA TAC CTA CTC CAT ATT CAG AGC GAA CGC GCC AAC
Thr Gln Ala Gly Glu Tyr Leu Leu His Ile Gln Ser Glu Arg Ala Asn
            140                 145                 150

TAC ACA GTA CTG TTC ACA GTG AAT GTA AGA GAT ACA CAG CTG TAT GTG
Tyr Thr Val Leu Phe Thr Val Asn Val Arg Asp Thr Gln Leu Tyr Val
        155                 160                 165

CTA AGG AGA CCT TAC TTT AGG AAG ATG GAA AAC CAG GAT GCA CTG CTC
Leu Arg Arg Pro Tyr Phe Arg Lys Met Glu Asn Gln Asp Ala Leu Leu
    170                 175                 180

TGC ATC TCC GAG GGT GTT CCG GAG CCC ACT GTG GAG TGG GTG CTC TGC
Cys Ile Ser Glu Gly Val Pro Glu Pro Thr Val Glu Trp Val Leu Cys
185                 190                 195                 200
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AGC TCC CAC AGG GAA AGC TGT AAA GAA GAA GGC CCT GCT GTT GTC AGA
Ser Ser His Arg Glu Ser Cys Lys Glu Glu Gly Pro Ala Val Val Arg
                205                 210                 215

AAG GAG GAA AAG GTA CTT CAT GAG TTG TTC GGA ACA GAC ATC AGA TGC
Lys Glu Glu Lys Val Leu His Glu Leu Phe Gly Thr Asp Ile Arg Cys
            220                 225                 230

TGT GCT AGA AAT GCA CTG GGC CGC GAA TGC ACC AAG CTG TTC ACC ATA
Cys Ala Arg Asn Ala Leu Gly Arg Glu Cys Thr Lys Leu Phe Thr Ile
        235                 240                 245

GAT CTA AAC CAG GCT CCT CAG AGC ACA CTG CCC CAG TTA TTC CTG AAA
Asp Leu Asn Gln Ala Pro Gln Ser Thr Leu Pro Gln Leu Phe Leu Lys
    250                 255                 260

GTG GGG GAA CCC TTG TGG ATC AGG TGT AAG GCC ATC CAT GTG AAC CAT
Val Gly Glu Pro Leu Trp Ile Arg Cys Lys Ala Ile His Val Asn His
265                 270                 275                 280

GGA TTC GGG CTC ACC TGG GAG CTG GAA GAC AAA GCC CTG GAG GAG GGC
Gly Phe Gly Leu Thr Trp Glu Leu Glu Asp Lys Ala Leu Glu Glu Gly
                285                 290                 295

AGC TAC TTT GAG ATG AGT ACC TAC TCC ACA AAC AGG ACC ATG ATT CGG
Ser Tyr Phe Glu Met Ser Thr Tyr Ser Thr Asn Arg Thr Met Ile Arg
            300                 305                 310

ATT CTC TTG GCC TTT GTG TCT TCC GTG GGA AGG AAC GAC ACC GGA TAT
Ile Leu Leu Ala Phe Val Ser Ser Val Gly Arg Asn Asp Thr Gly Tyr
        315                 320                 325

TAC ACC TGC TCT TCC TCA AAG CAC CCC AGC CAG TCA GCG TTG GTG ACC
Tyr Thr Cys Ser Ser Ser Lys His Pro Ser Gln Ser Ala Leu Val Thr
    330                 335                 340

ATC CTA GAA AAA GGG TTT ATA AAC GCT ACC AGC TCG CAA GAA GAG TAT
Ile Leu Glu Lys Gly Phe Ile Asn Ala Thr Ser Ser Gln Glu Glu Tyr
345                 350                 355                 360

GAA ATT GAC CCG TAC GAA AAG TTC TGC TTC TCA GTC AGG TTT AAA GCG
Glu Ile Asp Pro Tyr Glu Lys Phe Cys Phe Ser Val Arg Phe Lys Ala
                365                 370                 375

TAC CCA CGA ATC CGA TGC ACG TGG ATC TTC TCT CAA GCC TCA TTT CCT
Tyr Pro Arg Ile Arg Cys Thr Trp Ile Phe Ser Gln Ala Ser Phe Pro
            380                 385                 390

TGT GAA CAG AGA GGC CTG GAG GAT GGG TAC AGC ATA TCT AAA TTT TGC
Cys Glu Gln Arg Gly Leu Glu Asp Gly Tyr Ser Ile Ser Lys Phe Cys
        395                 400                 405

GAT CAT AAG AAC AAG CCA GGA GAG TAC ATA TTC TAT GCA GAA AAT GAT
Asp His Lys Asn Lys Pro Gly Glu Tyr Ile Phe Tyr Ala Glu Asn Asp
    410                 415                 420
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GAC GCC CAG TTC ACC AAA ATG TTC ACG CTG AAT ATA AGA AAG AAA CCT
Asp Ala Gln Phe Thr Lys Met Phe Thr Leu Asn Ile Arg Lys Lys Pro
425                 430                 435                 440

CAA GTG CTA GCA AAT GCC TCA GCC AGC CAG GCG TCC TGT TCC TCT GAT
Gln Val Leu Ala Asn Ala Ser Ala Ser Gln Ala Ser Cys Ser Ser Asp
                445                 450                 455

GGC TAC CCG CTA CCC TCT TGG ACC TGG AAG AAG TGT TCG GAC AAA TCT
Gly Tyr Pro Leu Pro Ser Trp Thr Trp Lys Lys Cys Ser Asp Lys Ser
            460                 465                 470

CCC AAT TGC ACG GAG GAA ATC CCA GAA GGA GTT TGG AAT AAA AAG GCT
Pro Asn Cys Thr Glu Glu Ile Pro Glu Gly Val Trp Asn Lys Lys Ala
        475                 480                 485

AAC AGA AAA GTG TTT GGC CAG TGG GTG TCG AGC AGT ACT CTA AAT ATG
Asn Arg Lys Val Phe Gly Gln Trp Val Ser Ser Ser Thr Leu Asn Met
    490                 495                 500

AGT GAG GCC GGG AAA GGG CTT CTG GTC AAA TGC TGT GCG TAC AAT TCT
Ser Glu Ala Gly Lys Gly Leu Leu Val Lys Cys Cys Ala Tyr Asn Ser
505                 510                 515                 520

ATG GGC ACG TCT TGC GAA ACC ATC TTT TTA AAC TCA CCA GGC CCC TTC
Met Gly Thr Ser Cys Glu Thr Ile Phe Leu Asn Ser Pro Gly Pro Phe
                525                 530                 535

CCT TTC ATC CAA GAC AAC ATC TCC TTC TAT GCG ACC ATT GGG CTC TGT
Pro Phe Ile Gln Asp Asn Ile Ser Phe Tyr Ala Thr Ile Gly Leu Cys
            540                 545                 550

CTC CCC TTC ATT GTT GTT CTC ATT GTG TTG ATC TGC CAC AAA TAC AAA
Leu Pro Phe Ile Val Val Leu Ile Val Leu Ile Cys His Lys Tyr Lys
        555                 560                 565

AAG CAA TTT AGG TAC GAG AGT CAG CTG CAG ATG ATC CAG GTG ACT GGC
Lys Gln Phe Arg Tyr Glu Ser Gln Leu Gln Met Ile Gln Val Thr Gly
    570                 575                 580

CCC CTG GAT AAC GAG TAC TTC TAC GTT GAC TTC AGG GAC TAT GAA TAT
Pro Leu Asp Asn Glu Tyr Phe Tyr Val Asp Phe Arg Asp Tyr Glu Tyr
585                 590                 595                 600

GAC CTT AAG TGG GAG TTC CCG AGA GAG AAC TTA GAG TTT GGG AAG GTC
Asp Leu Lys Trp Glu Phe Pro Arg Glu Asn Leu Glu Phe Gly Lys Val
                605                 610                 615

CTG GGG TCT GGC GCT TTC GGG AGG GTG ATG AAC GCC ACG GCC TAT GGC
Leu Gly Ser Gly Ala Phe Gly Arg Val Met Asn Ala Thr Ala Tyr Gly
            620                 625                 630

ATT AGT AAA ACG GGA GTC TCA ATT CAG GTG GCG GTG AAG ATG CTA AAA
Ile Ser Lys Thr Gly Val Ser Ile Gln Val Ala Val Lys Met Leu Lys
        635                 640                 645
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GAG AAA GCT GAC AGC TGT GAA AAA GAA GCT CTC ATG TCG GAG CTC AAA
Glu Lys Ala Asp Ser Cys Glu Lys Glu Ala Leu Met Ser Glu Leu Lys
    650                 655                 660

ATG ATG ACC CAC CTG GGA CAC CAT GAC AAC ATC GTG AAT CTG CTG GGG
Met Met Thr His Leu Gly His His Asp Asn Ile Val Asn Leu Leu Gly
665                 670                 675                 680

GCA TGC ACA CTG TCA GGG CCA GTG TAC TTG ATT TTT GAA TAT TGT TGC
Ala Cys Thr Leu Ser Gly Pro Val Tyr Leu Ile Phe Glu Tyr Cys Cys
                685                 690                 695

TAT GGT GAC CTC CTC AAC TAC CTA AGA AGT AAA AGA GAG AAG TTT CAC
Tyr Gly Asp Leu Leu Asn Tyr Leu Arg Ser Lys Arg Glu Lys Phe His
            700                 705                 710

AGG ACA TGG ACA GAG ATT TTT AAG GAA CAT AAT TTC AGT TCT TAC CCT
Arg Thr Trp Thr Glu Ile Phe Lys Glu His Asn Phe Ser Ser Tyr Pro
        715                 720                 725

ACT TTC CAG GCA CAT TCA AAT TCC AGC ATG CCT GGT TCA CGA GAA GTT
Thr Phe Gln Ala His Ser Asn Ser Ser Met Pro Gly Ser Arg Glu Val
    730                 735                 740

CAG TTA CAC CCG CCC TTG GAT CAG CTC TCA GGG TTC AAT GGG AAT TCA
Gln Leu His Pro Pro Leu Asp Gln Leu Ser Gly Phe Asn Gly Asn Ser
745                 750                 755                 760

ATT CAT TCT GAA GAT GAG ATT GAA TAT GAA AAC CAG AAG AGG CTG GCA
Ile His Ser Glu Asp Glu Ile Glu Tyr Glu Asn Gln Lys Arg Leu Ala
                765                 770                 775

GAA GAA GAG GAG GAA GAT TTG AAC GTG CTG ACG TTT GAA GAC CTC CTT
Glu Glu Glu Glu Glu Asp Leu Asn Val Leu Thr Phe Glu Asp Leu Leu
            780                 785                 790

TGC TTT GCG TAC CAA GTG GCC AAA GGC ATG GAA TTC CTG GAG TTC AAG
Cys Phe Ala Tyr Gln Val Ala Lys Gly Met Glu Phe Leu Glu Phe Lys
        795                 800                 805

TCG TGT GTC CAC AGA GAC CTG GCA GCC AGG AAT GTG TTG GTC ACC CAC
Ser Cys Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Thr His
    810                 815                 820

GGG AAG GTG GTG AAG ATC TGT GAC TTT GGA CTG GCC CGA GAC ATC CTG
Gly Lys Val Val Lys Ile Cys Asp Phe Gly Leu Ala Arg Asp Ile Leu
825                 830                 835                 840

AGC GAC TCC AGC TAC GTC GTC AGG GGC AAC GCA CGG CTG CCG GTG AAG
Ser Asp Ser Ser Tyr Val Val Arg Gly Asn Ala Arg Leu Pro Val Lys
                845                 850                 855

TGG ATG GCA CCC GAG AGC TTA TTT GAA GGG ATC TAC ACA ATC AAG AGT
Trp Met Ala Pro Glu Ser Leu Phe Glu Gly Ile Tyr Thr Ile Lys Ser
            860                 865                 870
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GAC GTC TGG TCC TAC GGC ATC CTT CTC TGG GAG ATA TTT TCA CTG GGT
Asp Val Trp Ser Tyr Gly Ile Leu Leu Trp Glu Ile Phe Ser Leu Gly
        875                 880                 885

GTG AAC CCT TAC CCT GGC ATT CCT GTC GAC GCT AAC TTC TAT AAA CTG
Val Asn Pro Tyr Pro Gly Ile Pro Val Asp Ala Asn Phe Tyr Lys Leu
    890                 895                 900

ATT CAG AGT GGA TTT AAA ATG GAG CAG CCA TTC TAT GCC ACA GAA GGG
Ile Gln Ser Gly Phe Lys Met Glu Gln Pro Phe Tyr Ala Thr Glu Gly
905                 910                 915                 920

ATA TAC TTT GTA ATG CAA TCC TGC TGG GCT TTT GAC TCA AGG AAG CGG
Ile Tyr Phe Val Met Gln Ser Cys Trp Ala Phe Asp Ser Arg Lys Arg
                925                 930                 935

CCA TCC TTC CCC AAC CTG ACT TCA TTT TTA GGA TGT CAG CTG GCA GAG
Pro Ser Phe Pro Asn Leu Thr Ser Phe Leu Gly Cys Gln Leu Ala Glu
            940                 945                 950

GCA GAA GAA GCA TGT ATC AGA ACA TCC ATC CAT CTA CCA AAA CAG GCG
Ala Glu Glu Ala Cys Ile Arg Thr Ser Ile His Leu Pro Lys Gln Ala
        955                 960                 965

GCC CCT CAG CAG AGA GGC GGG CTC AGA GCC CAG TCG CCA CAG CGC CAG
Ala Pro Gln Gln Arg Gly Gly Leu Arg Ala Gln Ser Pro Gln Arg Gln
    970                 975                 980

GTG AAG ATT CAC AGA GAA AGA AGT TAGCGAGGAG GCCTTGGACC CCGCCACCCT
Val Lys Ile His Arg Glu Arg Ser
985                 990

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CGTTGCTTCG CTGGACTTTT CTCTAGATGC TGTCTGCCAT TACTCCAAAG TGACTTCTAT

AAAATCAAAC CTCTCCTCGC ACAGGCGGGA GAGCCAATAA TGAGACTTGT TGGTGAGCCC

GCCTACCCTG GGGGCCTTTC CACGAGCTTG AGGGGAAAGC CATGTATCTG AAATATAGTA

TATTCTTGTA AATACGTGAA ACAAACCAAA CCCGTTTTTT GCTAAGGGAA AGCTAAATAT

GATTTTTAAA AATCTATGTT TTAAAATACT ATGTAACTTT TTCATCTATT TAGTGATATA

TTTTATGGAT GGAAATAAAC TTTCTACTGT AAAAAAAAAA AAAAAAAAAA AAAAAAA
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 992 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Arg Ala Leu Ala Gln Arg Ser Asp Arg Arg Leu Leu Leu Leu Val
1               5                   10                  15

Val Leu Ser Val Met Ile Leu Glu Thr Val Thr Asn Gln Asp Leu Pro
            20                  25                  30

Val Ile Lys Cys Val Leu Ile Ser His Glu Asn Asn Gly Ser Ser Ala
        35                  40                  45

Gly Lys Pro Ser Ser Tyr Arg Met Val Arg Gly Ser Pro Glu Asp Leu
    50                  55                  60

Gln Cys Thr Pro Arg Arg Gln Ser Glu Gly Thr Val Tyr Glu Ala Ala
65                  70                  75                  80

Thr Val Glu Val Ala Glu Ser Gly Ser Ile Thr Leu Gln Val Gln Leu
                85                  90                  95

Ala Thr Pro Gly Asp Leu Ser Cys Leu Trp Val Phe Lys His Ser Ser
            100                 105                 110

Leu Gly Cys Gln Pro His Phe Asp Leu Gln Asn Arg Gly Ile Val Ser
        115                 120                 125

Met Ala Ile Leu Asn Val Thr Glu Thr Gln Ala Gly Glu Tyr Leu Leu
    130                 135                 140

His Ile Gln Ser Glu Arg Ala Asn Tyr Thr Val Leu Phe Thr Val Asn
145                 150                 155                 160

Val Arg Asp Thr Gln Leu Tyr Val Leu Arg Arg Pro Tyr Phe Arg Lys
                165                 170                 175

Met Glu Asn Gln Asp Ala Leu Leu Cys Ile Ser Glu Gly Val Pro Glu
            180                 185                 190

Pro Thr Val Glu Trp Val Leu Cys Ser Ser His Arg Glu Ser Cys Lys
        195                 200                 205

Glu Glu Gly Pro Ala Val Val Arg Lys Glu Glu Lys Val Leu His Glu
    210                 215                 220

Leu Phe Gly Thr Asp Ile Arg Cys Cys Ala Arg Asn Ala Leu Gly Arg
225                 230                 235                 240

Glu Cys Thr Lys Leu Phe Thr Ile Asp Leu Asn Gln Ala Pro Gln Ser
                245                 250                 255

Thr Leu Pro Gln Leu Phe Leu Lys Val Gly Glu Pro Leu Trp Ile Arg
            260                 265                 270
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Cys Lys Ala Ile His Val Asn His Gly Phe Gly Leu Thr Trp Glu Leu
        275                 280                 285

Glu Asp Lys Ala Leu Glu Glu Gly Ser Tyr Phe Glu Met Ser Thr Tyr
    290                 295                 300

Ser Thr Asn Arg Thr Met Ile Arg Ile Leu Leu Ala Phe Val Ser Ser
305                 310                 315                     320

Val Gly Arg Asn Asp Thr Gly Tyr Tyr Thr Cys Ser Ser Ser Lys His
                325                 330                     335

Pro Ser Gln Ser Ala Leu Val Thr Ile Leu Glu Lys Gly Phe Ile Asn
            340                 345                 350

Ala Thr Ser Ser Gln Glu Glu Tyr Glu Ile Asp Pro Tyr Glu Lys Phe
        355                 360                 365

Cys Phe Ser Val Arg Phe Lys Ala Tyr Pro Arg Ile Arg Cys Thr Trp
    370                 375                 380

Ile Phe Ser Gln Ala Ser Phe Pro Cys Glu Gln Arg Gly Leu Glu Asp
385                 390                 395                     400

Gly Tyr Ser Ile Ser Lys Phe Cys Asp His Lys Asn Lys Pro Gly Glu
                405                 410                     415

Tyr Ile Phe Tyr Ala Glu Asn Asp Asp Ala Gln Phe Thr Lys Met Phe
            420                 425                 430

Thr Leu Asn Ile Arg Lys Lys Pro Gln Val Leu Ala Asn Ala Ser Ala
        435                 440                 445

Ser Gln Ala Ser Cys Ser Ser Asp Gly Tyr Pro Leu Pro Ser Trp Thr
    450                 455                 460

Trp Lys Lys Cys Ser Asp Lys Ser Pro Asn Cys Thr Glu Glu Ile Pro
465                 470                 475                     480

Glu Gly Val Trp Asn Lys Lys Ala Asn Arg Lys Val Phe Gly Gln Trp
                485                 490                     495

Val Ser Ser Ser Thr Leu Asn Met Ser Glu Ala Gly Lys Gly Leu Leu
            500                 505                 510

Val Lys Cys Cys Ala Tyr Asn Ser Met Gly Thr Ser Cys Glu Thr Ile
        515                 520                 525

Phe Leu Asn Ser Pro Gly Pro Phe Pro Phe Ile Gln Asp Asn Ile Ser
    530                 535                 540

Phe Tyr Ala Thr Ile Gly Leu Cys Leu Pro Phe Ile Val Val Leu Ile
545                 550                 555                     560

Val Leu Ile Cys His Lys Tyr Lys Lys Gln Phe Arg Tyr Glu Ser Gln
                565                 570                     575
```

Leu Gln Met Ile Gln Val Thr Gly Pro Leu Asp Asn Glu Tyr Phe Tyr
580 585 590

Val Asp Phe Arg Asp Tyr Glu Tyr Asp Leu Lys Trp Glu Phe Pro Arg
595 600 605

Glu Asn Leu Glu Phe Gly Lys Val Leu Gly Ser Gly Ala Phe Gly Arg
610 615 620

Val Met Asn Ala Thr Ala Tyr Gly Ile Ser Lys Thr Gly Val Ser Ile
625 630 635 640

Gln Val Ala Val Lys Met Leu Lys Glu Lys Ala Asp Ser Cys Glu Lys
645 650 655

Glu Ala Leu Met Ser Glu Leu Lys Met Met Thr His Leu Gly His His
660 665 670

Asp Asn Ile Val Asn Leu Leu Gly Ala Cys Thr Leu Ser Gly Pro Val
675 680 685

Tyr Leu Ile Phe Glu Tyr Cys Cys Tyr Gly Asp Leu Leu Asn Tyr Leu
690 695 700

Arg Ser Lys Arg Glu Lys Phe His Arg Thr Trp Thr Glu Ile Phe Lys
705 710 715 720

Glu His Asn Phe Ser Ser Tyr Pro Thr Phe Gln Ala His Ser Asn Ser
725 730 735

Ser Met Pro Gly Ser Arg Glu Val Gln Leu His Pro Pro Leu Asp Gln
740 745 750

Leu Ser Gly Phe Asn Gly Asn Ser Ile His Ser Glu Asp Glu Ile Glu
755 760 765

Tyr Glu Asn Gln Lys Arg Leu Ala Glu Glu Glu Glu Glu Asp Leu Asn
770 775 780

Val Leu Thr Phe Glu Asp Leu Leu Cys Phe Ala Tyr Gln Val Ala Lys
785 790 795 800

Gly Met Glu Phe Leu Glu Phe Lys Ser Cys Val His Arg Asp Leu Ala
805 810 815

Ala Arg Asn Val Leu Val Thr His Gly Lys Val Val Lys Ile Cys Asp
820 825 830

Phe Gly Leu Ala Arg Asp Ile Leu Ser Asp Ser Ser Tyr Val Val Arg
835 840 845

Gly Asn Ala Arg Leu Pro Val Lys Trp Met Ala Pro Glu Ser Leu Phe
850 855 860

Glu Gly Ile Tyr Thr Ile Lys Ser Asp Val Trp Ser Tyr Gly Ile Leu
865 870 875 880

```
Leu Trp Glu Ile Phe Ser Leu Gly Val Asn Pro Tyr Pro Gly Ile Pro
                885                 890                 895

Val Asp Ala Asn Phe Tyr Lys Leu Ile Gln Ser Gly Phe Lys Met Glu
            900                 905                 910

Gln Pro Phe Tyr Ala Thr Glu Gly Ile Tyr Phe Val Met Gln Ser Cys
        915                 920                 925

Trp Ala Phe Asp Ser Arg Lys Arg Pro Ser Phe Pro Asn Leu Thr Ser
    930                 935                 940

Phe Leu Gly Cys Gln Leu Ala Glu Ala Glu Glu Ala Cys Ile Arg Thr
945                 950                 955                 960

Ser Ile His Leu Pro Lys Gln Ala Ala Pro Gln Gln Arg Gly Gly Leu
                965                 970                 975

Arg Ala Gln Ser Pro Gln Arg Gln Val Lys Ile His Arg Glu Arg Ser
            980                 985                 990
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 332 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..332

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
AAC AAT GAT TCA TCA GTG GGG AAG TCA TCA TCA TAT CCC ATG GTA TCA
Asn Asn Asp Ser Ser Val Gly Lys Ser Ser Ser Tyr Pro Met Val Ser
  1               5                  10                  15

GAA TCC CCG GAA GAC CTC GGG TGT GCG TTG AGA CCC CAG AGC TCA GGG
Glu Ser Pro Glu Asp Leu Gly Cys Ala Leu Arg Pro Gln Ser Ser Gly
            20                  25                  30

ACA GTG TAC GAA GCT GCC GCT GTG GAA GTG GAT GTA TCT GCT TCC ATC
Thr Val Tyr Glu Ala Ala Ala Val Glu Val Asp Val Ser Ala Ser Ile
        35                  40                  45

ACA CTG CAA GTG CTG GTC GAT GCC CCA GGG AAC ATT TCC TGT CTC TGG
Thr Leu Gln Val Leu Val Asp Ala Pro Gly Asn Ile Ser Cys Leu Trp
    50                  55                  60
```

```
GTC TTT AAG CAC AGC TCC CTG AAT TGC CAG CCA CAT TTT GAT TTA CAA
Val Phe Lys His Ser Ser Leu Asn Cys Gln Pro His Phe Asp Leu Gln
 65                  70                  75                  80

AAC AGA GGA GTT GTT TCC ATG GTC ATT TTG AAA ATG ACA GAA ACC CAA
Asn Arg Gly Val Val Ser Met Val Ile Leu Lys Met Thr Glu Thr Gln
                 85                  90                  95

GCT GGA GAA TAC CTA CTT TTT ATT CAG AGT GAA GCT ACC AAT TA
Ala Gly Glu Tyr Leu Leu Phe Ile Gln Ser Glu Ala Thr Asn
            100                 105                 110
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 110 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Asn Asn Asp Ser Ser Val Gly Lys Ser Ser Ser Tyr Pro Met Val Ser
  1               5                  10                  15

Glu Ser Pro Glu Asp Leu Gly Cys Ala Leu Arg Pro Gln Ser Ser Gly
             20                  25                  30

Thr Val Tyr Glu Ala Ala Ala Val Glu Val Asp Val Ser Ala Ser Ile
         35                  40                  45

Thr Leu Gln Val Leu Val Asp Ala Pro Gly Asn Ile Ser Cys Leu Trp
     50                  55                  60

Val Phe Lys His Ser Ser Leu Asn Cys Gln Pro His Phe Asp Leu Gln
 65                  70                  75                  80

Asn Arg Gly Val Val Ser Met Val Ile Leu Lys Met Thr Glu Thr Gln
                 85                  90                  95

Ala Gly Glu Tyr Leu Leu Phe Ile Gln Ser Glu Ala Thr Asn
            100                 105                 110
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 284 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS

(B) LOCATION: 1..282

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
GAT CAA ATC TCA GGC TTC ATG GAA TTC ATT CAC TCT GAA GAT GAA ATT
Asp Gln Ile Ser Gly Phe Met Glu Phe Ile His Ser Glu Asp Glu Ile
  1               5                  10                  15

GAA TAT GAA AAC CAA AAA AAG AGG CTG GAA GAA GAG GAG GAC TTG AAT
Glu Tyr Glu Asn Gln Lys Lys Arg Leu Glu Glu Glu Glu Asp Leu Asn
             20                  25                  30

GTG CTT ACA TTT GAA GAT CTT CTT TGC TTT GCA TAT CAA GTT GCC AAA
Val Leu Thr Phe Glu Asp Leu Leu Cys Phe Ala Tyr Gln Val Ala Lys
         35                  40                  45

GGA ATG GAA TTT AAG TCG TGT GTT CAC AGA GAC CTG GCC GCC AGG AAC
Gly Met Glu Phe Lys Ser Cys Val His Arg Asp Leu Ala Ala Arg Asn
     50                  55                  60

GTG CTT GTC ACC CAC GGG AAA GTG GTG AAG ATA TGT GAC TTT GGA TTG
Val Leu Val Thr His Gly Lys Val Val Lys Ile Cys Asp Phe Gly Leu
 65                  70                  75                  80

GCT CGA GAT ATC ATG AGT GAT TCC GGC TAT GTT GTC AGG CAA
Ala Arg Asp Ile Met Ser Asp Ser Gly Tyr Val Val Arg Gln
                 85                  90

TC                                                             284
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 94 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Asp Gln Ile Ser Gly Phe Met Glu Phe Ile His Ser Glu Asp Glu Ile
  1               5                  10                  15

Glu Tyr Glu Asn Gln Lys Lys Arg Leu Glu Glu Glu Glu Asp Leu Asn
             20                  25                  30

Val Leu Thr Phe Glu Asp Leu Leu Cys Phe Ala Tyr Gln Val Ala Lys
         35                  40                  45

Gly Met Glu Phe Lys Ser Cys Val His Arg Asp Leu Ala Ala Arg Asn
     50                  55                  60

Val Leu Val Thr His Gly Lys Val Val Lys Ile Cys Asp Phe Gly Leu
 65                  70                  75                  80
```

```
Ala Arg Asp Ile Met Ser Asp Ser Gly Tyr Val Val Arg Gln
                85                  90
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5406 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 208..4311

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 208..4308

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
CTGTGTCCCG CAGCCGGATA ACCTGGCTGA CCCGATTCCG CGGACACCCG TGCAGCCGCG

GCTGGAGCCA GGGCGCCGGT GCCCGCGCTC TCCCCGGTCT TGCGCTGCGG GGGCCGATAC

CGCCTCTGTG ACTTCTTTGC GGGCCAGGGA CGGAGAAGGA GTCTGTGCCT GAGAAACTGG

GCTCTGTGCC CAGGCGCGAG GTGCAGG ATG GAG AGC AAG GGC CTG CTA GCT
                              Met Glu Ser Lys Gly Leu Leu Ala
                                1               5

GTC GCT CTG TGG TTC TGC GTG GAG ACC CGA GCC GCC TCT GTG GGT TTG
Val Ala Leu Trp Phe Cys Val Glu Thr Arg Ala Ala Ser Val Gly Leu
     10                  15                  20

CCT GGC GAT TTT CTC CAT CCC CCC AAG CTC AGC ACA CAG AAA GAC ATA
Pro Gly Asp Phe Leu His Pro Pro Lys Leu Ser Thr Gln Lys Asp Ile
 25                  30                  35                  40

CTG ACA ATT TTG GCA AAT ACA ACC CTT CAG ATT ACT TGC AGG GGA CAG
Leu Thr Ile Leu Ala Asn Thr Thr Leu Gln Ile Thr Cys Arg Gly Gln
                 45                  50                  55

CGG GAC CTG GAC TGG CTT TGG CCC AAT GCT CAG CGT GAT TCT GAG GAA
Arg Asp Leu Asp Trp Leu Trp Pro Asn Ala Gln Arg Asp Ser Glu Glu
             60                  65                  70

AGG GTA TTG GTG ACT GAA TGC GGC GGT GGT GAC AGT ATC TTC TGC AAA
Arg Val Leu Val Thr Glu Cys Gly Gly Gly Asp Ser Ile Phe Cys Lys
         75                  80                  85
```

ACA CTC ACC ATT CCC AGG GTG GTT GGA AAT GAT ACT GGA GCC TAC AAG
Thr Leu Thr Ile Pro Arg Val Val Gly Asn Asp Thr Gly Ala Tyr Lys
90 95 100

TGC TCG TAC CGG GAC GTC GAC ATA GCC TCC ACT GTT TAT GTC TAT GTT
Cys Ser Tyr Arg Asp Val Asp Ile Ala Ser Thr Val Tyr Val Tyr Val
105 110 115 120

CGA GAT TAC AGA TCA CCA TTC ATC GCC TCT GTC AGT GAC CAG CAT GGC
Arg Asp Tyr Arg Ser Pro Phe Ile Ala Ser Val Ser Asp Gln His Gly
125 130 135

ATC GTG TAC ATC ACC GAG AAC AAG AAC AAA ACT GTG GTG ATC CCC TGC
Ile Val Tyr Ile Thr Glu Asn Lys Asn Lys Thr Val Val Ile Pro Cys
140 145 150

CGA GGG TCG ATT TCA AAC CTC AAT GTG TCT CTT TGC GCT AGG TAT CCA
Arg Gly Ser Ile Ser Asn Leu Asn Val Ser Leu Cys Ala Arg Tyr Pro
155 160 165

GAA AAG AGA TTT GTT CCG GAT GGA AAC AGA ATT TCC TGG GAC AGC GAG
Glu Lys Arg Phe Val Pro Asp Gly Asn Arg Ile Ser Trp Asp Ser Glu
170 175 180

ATA GGC TTT ACT CTC CCC AGT TAC ATG ATC AGC TAT GCC GGC ATG GTC
Ile Gly Phe Thr Leu Pro Ser Tyr Met Ile Ser Tyr Ala Gly Met Val
185 190 195 200

TTC TGT GAG GCA AAG ATC AAT GAT GAA ACC TAT CAG TCT ATC ATG TAC
Phe Cys Glu Ala Lys Ile Asn Asp Glu Thr Tyr Gln Ser Ile Met Tyr
205 210 215

ATA GTT GTG GTT GTA GGA TAT AGG ATT TAT GAT GTG ATT CTG AGC CCC
Ile Val Val Val Val Gly Tyr Arg Ile Tyr Asp Val Ile Leu Ser Pro
220 225 230

CCG CAT GAA ATT GAG CTA TCT GCC GGA GAA AAA CTT GTC TTA AAT TGT
Pro His Glu Ile Glu Leu Ser Ala Gly Glu Lys Leu Val Leu Asn Cys
235 240 245

ACA GCG AGA ACA GAG CTC AAT GTG GGG CTT GAT TTC ACC TGG CAC TCT
Thr Ala Arg Thr Glu Leu Asn Val Gly Leu Asp Phe Thr Trp His Ser
250 255 260

CCA CCT TCA AAG TCT CAT CAT AAG AAG ATT GTA AAC CGG GAT GTG AAA
Pro Pro Ser Lys Ser His His Lys Lys Ile Val Asn Arg Asp Val Lys
265 270 275 280

CCC TTT CCT GGG ACT GTG GCG AAG ATG TTT TTG AGC ACC TTG ACA ATA
Pro Phe Pro Gly Thr Val Ala Lys Met Phe Leu Ser Thr Leu Thr Ile
285 290 295

GAA AGT GTG ACC AAG AGT GAC CAA GGG GAA TAC ACC TGT GTA GCG TCC
Glu Ser Val Thr Lys Ser Asp Gln Gly Glu Tyr Thr Cys Val Ala Ser
300 305 310

```
AGT GGA CGG ATG ATC AAG AGA AAT AGA ACA TTT GTC CGA GTT CAC ACA
Ser Gly Arg Met Ile Lys Arg Asn Arg Thr Phe Val Arg Val His Thr
        315                 320                 325

AAG CCT TTT ATT GCT TTC GGT AGT GGG ATG AAA TCT TTG GTG GAA GCC
Lys Pro Phe Ile Ala Phe Gly Ser Gly Met Lys Ser Leu Val Glu Ala
    330                 335                 340

ACA GTG GGC AGT CAA GTC CGA ATC CCT GTG AAG TAT CTC AGT TAC CCA
Thr Val Gly Ser Gln Val Arg Ile Pro Val Lys Tyr Leu Ser Tyr Pro
345                 350                 355                 360

GCT CCT GAT ATC AAA TGG TAC AGA AAT GGA AGG CCC ATT GAG TCC AAC
Ala Pro Asp Ile Lys Trp Tyr Arg Asn Gly Arg Pro Ile Glu Ser Asn
                365                 370                 375

TAC ACA ATG ATT GTT GGC GAT GAA CTC ACC ATC ATG GAA GTG ACT GAA
Tyr Thr Met Ile Val Gly Asp Glu Leu Thr Ile Met Glu Val Thr Glu
            380                 385                 390

AGA GAT GCA GGA AAC TAC ACG GTC ATC CTC ACC AAC CCC ATT TCA ATG
Arg Asp Ala Gly Asn Tyr Thr Val Ile Leu Thr Asn Pro Ile Ser Met
        395                 400                 405

GAG AAA CAG AGC CAC ATG GTC TCT CTG GTT GTG AAT GTC CCA CCC CAG
Glu Lys Gln Ser His Met Val Ser Leu Val Val Asn Val Pro Pro Gln
    410                 415                 420

ATC GGT GAG AAA GCC TTG ATC TCG CCT ATG GAT TCC TAC CAG TAT GGG
Ile Gly Glu Lys Ala Leu Ile Ser Pro Met Asp Ser Tyr Gln Tyr Gly
425                 430                 435                 440

ACC ATG CAG ACA TTG ACA TGC ACA GTC TAC GCC AAC CCT CCC CTG CAC
Thr Met Gln Thr Leu Thr Cys Thr Val Tyr Ala Asn Pro Pro Leu His
                445                 450                 455

CAC ATC CAG TGG TAC TGG CAG CTA GAA GAA GCC TGC TCC TAC AGA CCC
His Ile Gln Trp Tyr Trp Gln Leu Glu Glu Ala Cys Ser Tyr Arg Pro
            460                 465                 470

GGC CAA ACA AGC CCG TAT GCT TGT AAA GAA TGG AGA CAC GTG GAG GAT
Gly Gln Thr Ser Pro Tyr Ala Cys Lys Glu Trp Arg His Val Glu Asp
        475                 480                 485

TTC CAG GGG GGA AAC AAG ATC GAA GTC ACC AAA AAC CAA TAT GCC CTG
Phe Gln Gly Gly Asn Lys Ile Glu Val Thr Lys Asn Gln Tyr Ala Leu
    490                 495                 500

ATT GAA GGA AAA AAC AAA ACT GTA AGT ACG CTG GTC ATC CAA GCT GCC
Ile Glu Gly Lys Asn Lys Thr Val Ser Thr Leu Val Ile Gln Ala Ala
505                 510                 515                 520

AAC GTG TCA GCG TTG TAC AAA TGT GAA GCC ATC AAC AAA GCG GGA CGA
Asn Val Ser Ala Leu Tyr Lys Cys Glu Ala Ile Asn Lys Ala Gly Arg
                525                 530                 535
```

```
GGA GAG AGG GTC ATC TCC TTC CAT GTG ATC AGG GGT CCT GAA ATT ACT
Gly Glu Arg Val Ile Ser Phe His Val Ile Arg Gly Pro Glu Ile Thr
            540                 545                 550

GTG CAA CCT GCT GCC CAG CCA ACT GAG CAG GAG AGT GTG TCC CTG TTG
Val Gln Pro Ala Ala Gln Pro Thr Glu Gln Glu Ser Val Ser Leu Leu
        555                 560                 565

TGC ACT GCA GAC AGA AAT ACG TTT GAG AAC CTC ACG TGG TAC AAG CTT
Cys Thr Ala Asp Arg Asn Thr Phe Glu Asn Leu Thr Trp Tyr Lys Leu
    570                 575                 580

GGC TCA CAG GCA ACA TCG GTC CAC ATG GGC GAA TCA CTC ACA CCA GTT
Gly Ser Gln Ala Thr Ser Val His Met Gly Glu Ser Leu Thr Pro Val
585                 590                 595                 600

TGC AAG AAC TTG GAT GCT CTT TGG AAA CTG AAT GGC ACC ATG TTT TCT
Cys Lys Asn Leu Asp Ala Leu Trp Lys Leu Asn Gly Thr Met Phe Ser
                605                 610                 615

AAC AGC ACA AAT GAC ATC TTG ATT GTG GCA TTT CAG AAT GCC TCT CTG
Asn Ser Thr Asn Asp Ile Leu Ile Val Ala Phe Gln Asn Ala Ser Leu
            620                 625                 630

CAG GAC CAA GGC GAC TAT GTT TGC TCT GCT CAA GAT AAG AAG ACC AAG
Gln Asp Gln Gly Asp Tyr Val Cys Ser Ala Gln Asp Lys Lys Thr Lys
        635                 640                 645

AAA AGA CAT TGC CTG GTC AAA CAG CTC ATC ATC CTA GAG CGC ATG GCA
Lys Arg His Cys Leu Val Lys Gln Leu Ile Ile Leu Glu Arg Met Ala
    650                 655                 660

CCC ATG ATC ACC GGA AAT CTG GAG AAT CAG ACA ACA ACC ATT GGC GAG
Pro Met Ile Thr Gly Asn Leu Glu Asn Gln Thr Thr Thr Ile Gly Glu
665                 670                 675                 680

ACC ATT GAA GTG ACT TGC CCA GCA TCT GGA AAT CCT ACC CCA CAC ATT
Thr Ile Glu Val Thr Cys Pro Ala Ser Gly Asn Pro Thr Pro His Ile
                685                 690                 695

ACA TGG TTC AAA GAC AAC GAG ACC CTG GTA GAA GAT TCA GGC ATT GTA
Thr Trp Phe Lys Asp Asn Glu Thr Leu Val Glu Asp Ser Gly Ile Val
            700                 705                 710

CTG AGA GAT GGG AAC CGG AAC CTG ACT ATC CGC AGG GTG AGG AAG GAG
Leu Arg Asp Gly Asn Arg Asn Leu Thr Ile Arg Arg Val Arg Lys Glu
        715                 720                 725

GAT GGA GGC CTC TAC ACC TGC CAG GCC TGC AAT GTC CTT GGC TGT GCA
Asp Gly Gly Leu Tyr Thr Cys Gln Ala Cys Asn Val Leu Gly Cys Ala
    730                 735                 740

AGA GCG GAG ACG CTC TTC ATA ATA GAA GGT GCC CAG GAA AAG ACC AAC
Arg Ala Glu Thr Leu Phe Ile Ile Glu Gly Ala Gln Glu Lys Thr Asn
745                 750                 755                 760
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TTG GAA GTC ATT ATC CTC GTC GGC ACT GCA GTG ATT GCC ATG TTC TTC
Leu Glu Val Ile Ile Leu Val Gly Thr Ala Val Ile Ala Met Phe Phe
                765                 770                 775

TGG CTC CTT CTT GTC ATT CTC GTA CGG ACC GTT AAG CGG GCC AAT GAA
Trp Leu Leu Leu Val Ile Leu Val Arg Thr Val Lys Arg Ala Asn Glu
            780                 785                 790

GGG GAA CTG AAG ACA GGC TAC TTG TCT ATT GTC ATG GAT CCA GAT GAA
Gly Glu Leu Lys Thr Gly Tyr Leu Ser Ile Val Met Asp Pro Asp Glu
        795                 800                 805

TTG CCC TTG GAT GAG CGC TGT GAA CGC TTG CCT TAT GAT GCC AGC AAG
Leu Pro Leu Asp Glu Arg Cys Glu Arg Leu Pro Tyr Asp Ala Ser Lys
    810                 815                 820

TGG GAA TTC CCC AGG GAC CGG CTG AAA CTA GGA AAA CCT CTT GGC CGC
Trp Glu Phe Pro Arg Asp Arg Leu Lys Leu Gly Lys Pro Leu Gly Arg
825                 830                 835                 840

GGT GCC TTC GGC CAA GTG ATT GAG GCA GAC GCT TTT GGA ATT GAC AAG
Gly Ala Phe Gly Gln Val Ile Glu Ala Asp Ala Phe Gly Ile Asp Lys
                845                 850                 855

ACA GCG ACT TGC AAA ACA GTA GCC GTC AAG ATG TTG AAA GAA GGA GCA
Thr Ala Thr Cys Lys Thr Val Ala Val Lys Met Leu Lys Glu Gly Ala
            860                 865                 870

ACA CAC AGC GAG CAT CGA GCC CTC ATG TCT GAA CTC AAG ATC CTC ATC
Thr His Ser Glu His Arg Ala Leu Met Ser Glu Leu Lys Ile Leu Ile
        875                 880                 885

CAC ATT GGT CAC CAT CTC AAT GTG GTG AAC CTC CTA GGC GCC TGC ACC
His Ile Gly His His Leu Asn Val Val Asn Leu Leu Gly Ala Cys Thr
    890                 895                 900

AAG CCG GGA GGG CCT CTC ATG GTG ATT GTG GAA TTC TCG AAG TTT GGA
Lys Pro Gly Gly Pro Leu Met Val Ile Val Glu Phe Ser Lys Phe Gly
905                 910                 915                 920

AAC CTA TCA ACT TAC TTA CGG GGC AAG AGA AAT GAA TTT GTT CCC TAT
Asn Leu Ser Thr Tyr Leu Arg Gly Lys Arg Asn Glu Phe Val Pro Tyr
                925                 930                 935

AAG AGC AAA GGG GCA CGC TTC CGC CAG GGC AAG GAC TAC GTT GGG GAG
Lys Ser Lys Gly Ala Arg Phe Arg Gln Gly Lys Asp Tyr Val Gly Glu
            940                 945                 950

CTC TCC GTG GAT CTG AAA AGA CGC TTG GAC AGC ATC ACC AGC AGC CAG
Leu Ser Val Asp Leu Lys Arg Arg Leu Asp Ser Ile Thr Ser Ser Gln
        955                 960                 965

AGC TCT GCC AGC TCA GGC TTT GTT GAG GAG AAA TCG CTC AGT GAT GTA
Ser Ser Ala Ser Ser Gly Phe Val Glu Glu Lys Ser Leu Ser Asp Val
    970                 975                 980
```

GAG GAA GAA GAA GCT TCT GAA GAA CTG TAC AAG GAC TTC CTG ACC TTG
Glu Glu Glu Glu Ala Ser Glu Glu Leu Tyr Lys Asp Phe Leu Thr Leu
985 990 995 1000

GAG CAT CTC ATC TGT TAC AGC TTC CAA GTG GCT AAG GGC ATG GAG TTC
Glu His Leu Ile Cys Tyr Ser Phe Gln Val Ala Lys Gly Met Glu Phe
1005 1010 1015

TTG GCA TCA AGG AAG TGT ATC CAC AGG GAC CTG GCA GCA CGA AAC ATT
Leu Ala Ser Arg Lys Cys Ile His Arg Asp Leu Ala Ala Arg Asn Ile
1020 1025 1030

CTC CTA TCG GAG AAG AAT GTG GTT AAG ATC TGT GAC TTC GGC TTG GCC
Leu Leu Ser Glu Lys Asn Val Val Lys Ile Cys Asp Phe Gly Leu Ala
1035 1040 1045

CGG GAC ATT TAT AAA GAC CCG GAT TAT GTC AGA AAA GGA GAT GCC CGA
Arg Asp Ile Tyr Lys Asp Pro Asp Tyr Val Arg Lys Gly Asp Ala Arg
1050 1055 1060

CTC CCT TTG AAG TGG ATG GCC CCG GAA ACC ATT TTT GAC AGA GTA TAC
Leu Pro Leu Lys Trp Met Ala Pro Glu Thr Ile Phe Asp Arg Val Tyr
1065 1070 1075 1080

ACA ATT CAG AGC GAT GTG TGG TCT TTC GGT GTG TTG CTC TGG GAA ATA
Thr Ile Gln Ser Asp Val Trp Ser Phe Gly Val Leu Leu Trp Glu Ile
1085 1090 1095

TTT TCC TTA GGT GCC TCC CCA TAC CCT GGG GTC AAG ATT GAT GAA GAA
Phe Ser Leu Gly Ala Ser Pro Tyr Pro Gly Val Lys Ile Asp Glu Glu
1100 1105 1110

TTT TGT AGG AGA TTG AAA GAA GGA ACT AGA ATG CGG GCT CCT GAC TAC
Phe Cys Arg Arg Leu Lys Glu Gly Thr Arg Met Arg Ala Pro Asp Tyr
1115 1120 1125

ACT ACC CCA GAA ATG TAC CAG ACC ATG CTG GAC TGC TGG CAT GAG GAC
Thr Thr Pro Glu Met Tyr Gln Thr Met Leu Asp Cys Trp His Glu Asp
1130 1135 1140

CCC AAC CAG AGA CCC TCG TTT TCA GAG TTG GTG GAG CAT TTG GGA AAC
Pro Asn Gln Arg Pro Ser Phe Ser Glu Leu Val Glu His Leu Gly Asn
1145 1150 1155 1160

CTC CTG CAA GCA AAT GCG CAG CAG GAT GGC AAA GAC TAT ATT GTT CTT
Leu Leu Gln Ala Asn Ala Gln Gln Asp Gly Lys Asp Tyr Ile Val Leu
1165 1170 1175

CCA ATG TCA GAG ACA CTG AGC ATG GAA GAG GAT TCT GGA CTC TCC CTG
Pro Met Ser Glu Thr Leu Ser Met Glu Glu Asp Ser Gly Leu Ser Leu
1180 1185 1190

CCT ACC TCA CCT GTT TCC TGT ATG GAG GAA GAG GAA GTG TGC GAC CCC
Pro Thr Ser Pro Val Ser Cys Met Glu Glu Glu Glu Val Cys Asp Pro
1195 1200 1205

```
AAA TTC CAT TAT GAC AAC ACA GCA GGA ATC AGT CAT TAT CTC CAG AAC
Lys Phe His Tyr Asp Asn Thr Ala Gly Ile Ser His Tyr Leu Gln Asn
    1210                1215                1220

AGT AAG CGA AAG AGC CGG CCA GTG AGT GTA AAA ACA TTT GAA GAT ATC
Ser Lys Arg Lys Ser Arg Pro Val Ser Val Lys Thr Phe Glu Asp Ile
1225                1230                1235                1240

CCA TTG GAG GAA CCA GAA GTA AAA GTG ATC CCA GAT GAC AGC CAG ACA
Pro Leu Glu Glu Pro Glu Val Lys Val Ile Pro Asp Asp Ser Gln Thr
                1245                1250                1255

GAC AGT GGG ATG GTC CTT GCA TCA GAA GAG CTG AAA ACT CTG GAA GAC
Asp Ser Gly Met Val Leu Ala Ser Glu Glu Leu Lys Thr Leu Glu Asp
            1260                1265                1270

AGG AAC AAA TTA TCT CCA TCT TTT GGT GGA ATG ATG CCC AGT AAA AGC
Arg Asn Lys Leu Ser Pro Ser Phe Gly Gly Met Met Pro Ser Lys Ser
        1275                1280                1285

AGG GAG TCT GTG GCC TCG GAA GGC TCC AAC CAG ACC AGT GGC TAC CAG
Arg Glu Ser Val Ala Ser Glu Gly Ser Asn Gln Thr Ser Gly Tyr Gln
    1290                1295                1300

TCT GGG TAT CAC TCA GAT GAC ACA GAC ACC ACC GTG TAC TCC AGC GAC
Ser Gly Tyr His Ser Asp Asp Thr Asp Thr Thr Val Tyr Ser Ser Asp
1305                1310                1315                1320

GAG GCA GGA CTT TTA AAG ATG GTG GAT GCT GCA GTT CAC GCT GAC TCA
Glu Ala Gly Leu Leu Lys Met Val Asp Ala Ala Val His Ala Asp Ser
                1325                1330                1335

GGG ACC ACA CTG CAG CTC ACC TCC TGT TTA AAT GGA AGT GGT CCT GTC
Gly Thr Thr Leu Gln Leu Thr Ser Cys Leu Asn Gly Ser Gly Pro Val
            1340                1345                1350

CCG GCT CCG CCC CCA ACT CCT GGA AAT CAC GAG AGA GGT GCT GCT
Pro Ala Pro Pro Pro Thr Pro Gly Asn His Glu Arg Gly Ala Ala
        1355                1360                1365

TAGATTTTCA AGTGTTGTTC TTTCCACCAC CCGGAAGTAG CCACATTTGA TTTTCATTTT

TGGAGGAGGG ACCTCAGACT GCAAGGAGCT TGTCCTCAGG GCATTTCCAG AGAAGATGCC

CATGACCCAA GAATGTGTTG ACTCTACTCT CTTTTCCATT CATTTAAAAG TCCTATATAA

TGTGGTCTCA CTACCAGTTA AAGCAAAAGA CTTTCAAACA CGTGGACTCT GTCCTCCAAG

TGTGCCCTGC AAGTGGCAAC GGCACCTCTG TGAAACTGGA TCGAATGGGC AATGCTTTGT

GTGTTGAGGA TGGGTGAGAT GTCCCAGGGC CGAGTCTGTC TACCTTGGAG GCTTTGTGGA

GGATGCGGCT ATGAGCCAAG TGTTAAGTGT GGGATGTGGA CTGGGAGGAA GGAAGGCGCA

AGAGCGGTTG GAGCCTGCAG ATGCATTGTG CTGGCTCTGG TGGAGGTGGG CTTGTGGCCT
```

GTCAGGAAAC GCAAAGGCGG CCGGCAGGGT TTGGTTTTGG AAGGTTTGCG TGCTCTTCAC

AGTCGGGTTA CAGGCGAGTT CCCTGTGGCG TTTCCTACTC CTAATGAGAG TTCCTTCCGG

ACTCTTACGT GTCTCCTGGC CTGGCCCCAG GAAGGAAATG ATGCAGCTTG CTCCTTCCTC

ATCTCTCAGG CTGTGCCTTA ATTCAGAACA CCAAAAGAGA GGAACGTCGG CAGAGGCTCC

TGACGGGGCC GAAGAATTGT GAGAACAGAA CAGAAACTCA GGGTTTCTGC TGGGTGGAGA

CCCACGTGGC GCCCTGGTGG CAGGTCTGAG GGTTCTCTGT CAAGTGGCGG TAAAGGCTCA

GGCTGGTGTT CTTCCTCTAT CTCCACTCCT GTCAGGCCCC CAAGTCCTCA GTATTTTAGC

TTTGTGGCTT CCTGATGGCA GAAAAATCTT AATTGGTTGG TTTGCTCTCC AGATAATCAC

TAGCCAGATT TCGAAATTAC TTTTTAGCCG AGGTTATGAT AACATCTACT GTATCCTTTA

GAATTTTAAC CTATAAAACT ATGTCTACTG GTTTCTGCCT GTGTGCTTAT GTTAAAAAAA

AGCCGTCCGG AAAAAAAA 5406

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1367 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Glu Ser Lys Gly Leu Leu Ala Val Ala Leu Trp Phe Cys Val Glu
1 5 10 15

Thr Arg Ala Ala Ser Val Gly Leu Pro Gly Asp Phe Leu His Pro Pro
20 25 30

Lys Leu Ser Thr Gln Lys Asp Ile Leu Thr Ile Leu Ala Asn Thr Thr
35 40 45

Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp Trp Leu Trp Pro
50 55 60

Asn Ala Gln Arg Asp Ser Glu Glu Arg Val Leu Val Thr Glu Cys Gly
65 70 75 80

Gly Gly Asp Ser Ile Phe Cys Lys Thr Leu Thr Ile Pro Arg Val Val
85 90 95

Gly Asn Asp Thr Gly Ala Tyr Lys Cys Ser Tyr Arg Asp Val Asp Ile
100 105 110

Ala Ser Thr Val Tyr Val Tyr Val Arg Asp Tyr Arg Ser Pro Phe Ile
115 120 125

Ala Ser Val Ser Asp Gln His Gly Ile Val Tyr Ile Thr Glu Asn Lys
    130   135   140

Asn Lys Thr Val Val Ile Pro Cys Arg Gly Ser Ile Ser Asn Leu Asn
145   150   155   160

Val Ser Leu Cys Ala Arg Tyr Pro Glu Lys Arg Phe Val Pro Asp Gly
                165   170   175

Asn Arg Ile Ser Trp Asp Ser Glu Ile Gly Phe Thr Leu Pro Ser Tyr
            180   185   190

Met Ile Ser Tyr Ala Gly Met Val Phe Cys Glu Ala Lys Ile Asn Asp
        195   200   205

Glu Thr Tyr Gln Ser Ile Met Tyr Ile Val Val Val Val Gly Tyr Arg
    210   215   220

Ile Tyr Asp Val Ile Leu Ser Pro Pro His Glu Ile Glu Leu Ser Ala
225   230   235   240

Gly Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val
                245   250   255

Gly Leu Asp Phe Thr Trp His Ser Pro Pro Ser Lys Ser His His Lys
            260   265   270

Lys Ile Val Asn Arg Asp Val Lys Pro Phe Pro Gly Thr Val Ala Lys
        275   280   285

Met Phe Leu Ser Thr Leu Thr Ile Glu Ser Val Thr Lys Ser Asp Gln
    290   295   300

Gly Glu Tyr Thr Cys Val Ala Ser Ser Gly Arg Met Ile Lys Arg Asn
305   310   315   320

Arg Thr Phe Val Arg Val His Thr Lys Pro Phe Ile Ala Phe Gly Ser
                325   330   335

Gly Met Lys Ser Leu Val Glu Ala Thr Val Gly Ser Gln Val Arg Ile
            340   345   350

Pro Val Lys Tyr Leu Ser Tyr Pro Ala Pro Asp Ile Lys Trp Tyr Arg
        355   360   365

Asn Gly Arg Pro Ile Glu Ser Asn Tyr Thr Met Ile Val Gly Asp Glu
    370   375   380

Leu Thr Ile Met Glu Val Thr Glu Arg Asp Ala Gly Asn Tyr Thr Val
385   390   395   400

Ile Leu Thr Asn Pro Ile Ser Met Glu Lys Gln Ser His Met Val Ser
                405   410   415

Leu Val Val Asn Val Pro Pro Gln Ile Gly Glu Lys Ala Leu Ile Ser
            420   425   430

Pro Met Asp Ser Tyr Gln Tyr Gly Thr Met Gln Thr Leu Thr Cys Thr
435 440 445

Val Tyr Ala Asn Pro Pro Leu His His Ile Gln Trp Tyr Trp Gln Leu
450 455 460

Glu Glu Ala Cys Ser Tyr Arg Pro Gly Gln Thr Ser Pro Tyr Ala Cys
465 470 475 480

Lys Glu Trp Arg His Val Glu Asp Phe Gln Gly Gly Asn Lys Ile Glu
485 490 495

Val Thr Lys Asn Gln Tyr Ala Leu Ile Glu Gly Lys Asn Lys Thr Val
500 505 510

Ser Thr Leu Val Ile Gln Ala Ala Asn Val Ser Ala Leu Tyr Lys Cys
515 520 525

Glu Ala Ile Asn Lys Ala Gly Arg Gly Glu Arg Val Ile Ser Phe His
530 535 540

Val Ile Arg Gly Pro Glu Ile Thr Val Gln Pro Ala Ala Gln Pro Thr
545 550 555 560

Glu Gln Glu Ser Val Ser Leu Leu Cys Thr Ala Asp Arg Asn Thr Phe
565 570 575

Glu Asn Leu Thr Trp Tyr Lys Leu Gly Ser Gln Ala Thr Ser Val His
580 585 590

Met Gly Glu Ser Leu Thr Pro Val Cys Lys Asn Leu Asp Ala Leu Trp
595 600 605

Lys Leu Asn Gly Thr Met Phe Ser Asn Ser Thr Asn Asp Ile Leu Ile
610 615 620

Val Ala Phe Gln Asn Ala Ser Leu Gln Asp Gln Gly Asp Tyr Val Cys
625 630 635 640

Ser Ala Gln Asp Lys Lys Thr Lys Lys Arg His Cys Leu Val Lys Gln
645 650 655

Leu Ile Ile Leu Glu Arg Met Ala Pro Met Ile Thr Gly Asn Leu Glu
660 665 670

Asn Gln Thr Thr Thr Ile Gly Glu Thr Ile Glu Val Thr Cys Pro Ala
675 680 685

Ser Gly Asn Pro Thr Pro His Ile Thr Trp Phe Lys Asp Asn Glu Thr
690 695 700

Leu Val Glu Asp Ser Gly Ile Val Leu Arg Asp Gly Asn Arg Asn Leu
705 710 715 720

Thr Ile Arg Arg Val Arg Lys Glu Asp Gly Gly Leu Tyr Thr Cys Gln
725 730 735

Ala Cys Asn Val Leu Gly Cys Ala Arg Ala Glu Thr Leu Phe Ile Ile
740 745 750

Glu Gly Ala Gln Glu Lys Thr Asn Leu Glu Val Ile Ile Leu Val Gly
755 760 765

Thr Ala Val Ile Ala Met Phe Phe Trp Leu Leu Leu Val Ile Leu Val
770 775 780

Arg Thr Val Lys Arg Ala Asn Glu Gly Glu Leu Lys Thr Gly Tyr Leu
785 790 795 800

Ser Ile Val Met Asp Pro Asp Glu Leu Pro Leu Asp Glu Arg Cys Glu
805 810 815

Arg Leu Pro Tyr Asp Ala Ser Lys Trp Glu Phe Pro Arg Asp Arg Leu
820 825 830

Lys Leu Gly Lys Pro Leu Gly Arg Gly Ala Phe Gly Gln Val Ile Glu
835 840 845

Ala Asp Ala Phe Gly Ile Asp Lys Thr Ala Thr Cys Lys Thr Val Ala
850 855 860

Val Lys Met Leu Lys Glu Gly Ala Thr His Ser Glu His Arg Ala Leu
865 870 875 880

Met Ser Glu Leu Lys Ile Leu Ile His Ile Gly His His Leu Asn Val
885 890 895

Val Asn Leu Leu Gly Ala Cys Thr Lys Pro Gly Gly Pro Leu Met Val
900 905 910

Ile Val Glu Phe Ser Lys Phe Gly Asn Leu Ser Thr Tyr Leu Arg Gly
915 920 925

Lys Arg Asn Glu Phe Val Pro Tyr Lys Ser Lys Gly Ala Arg Phe Arg
930 935 940

Gln Gly Lys Asp Tyr Val Gly Glu Leu Ser Val Asp Leu Lys Arg Arg
945 950 955 960

Leu Asp Ser Ile Thr Ser Ser Gln Ser Ser Ala Ser Ser Gly Phe Val
965 970 975

Glu Glu Lys Ser Leu Ser Asp Val Glu Glu Glu Glu Ala Ser Glu Glu
980 985 990

Leu Tyr Lys Asp Phe Leu Thr Leu Glu His Leu Ile Cys Tyr Ser Phe
995 1000 1005

Gln Val Ala Lys Gly Met Glu Phe Leu Ala Ser Arg Lys Cys Ile His
1010 1015 1020

Arg Asp Leu Ala Ala Arg Asn Ile Leu Leu Ser Glu Lys Asn Val Val
1025 1030 1035 1040

Lys Ile Cys Asp Phe Gly Leu Ala Arg Asp Ile Tyr Lys Asp Pro Asp
1045 1050 1055

Tyr Val Arg Lys Gly Asp Ala Arg Leu Pro Leu Lys Trp Met Ala Pro
1060 1065 1070

Glu Thr Ile Phe Asp Arg Val Tyr Thr Ile Gln Ser Asp Val Trp Ser
1075 1080 1085

Phe Gly Val Leu Leu Trp Glu Ile Phe Ser Leu Gly Ala Ser Pro Tyr
1090 1095 1100

Pro Gly Val Lys Ile Asp Glu Glu Phe Cys Arg Arg Leu Lys Glu Gly
1105 1110 1115 1120

Thr Arg Met Arg Ala Pro Asp Tyr Thr Thr Pro Glu Met Tyr Gln Thr
1125 1130 1135

Met Leu Asp Cys Trp His Glu Asp Pro Asn Gln Arg Pro Ser Phe Ser
1140 1145 1150

Glu Leu Val Glu His Leu Gly Asn Leu Leu Gln Ala Asn Ala Gln Gln
1155 1160 1165

Asp Gly Lys Asp Tyr Ile Val Leu Pro Met Ser Glu Thr Leu Ser Met
1170 1175 1180

Glu Glu Asp Ser Gly Leu Ser Leu Pro Thr Ser Pro Val Ser Cys Met
1185 1190 1195 1200

Glu Glu Glu Glu Val Cys Asp Pro Lys Phe His Tyr Asp Asn Thr Ala
1205 1210 1215

Gly Ile Ser His Tyr Leu Gln Asn Ser Lys Arg Lys Ser Arg Pro Val
1220 1225 1230

Ser Val Lys Thr Phe Glu Asp Ile Pro Leu Glu Glu Pro Glu Val Lys
1235 1240 1245

Val Ile Pro Asp Asp Ser Gln Thr Asp Ser Gly Met Val Leu Ala Ser
1250 1255 1260

Glu Glu Leu Lys Thr Leu Glu Asp Arg Asn Lys Leu Ser Pro Ser Phe
1265 1270 1275 1280

Gly Gly Met Met Pro Ser Lys Ser Arg Glu Ser Val Ala Ser Glu Gly
1285 1290 1295

Ser Asn Gln Thr Ser Gly Tyr Gln Ser Gly Tyr His Ser Asp Asp Thr
1300 1305 1310

Asp Thr Thr Val Tyr Ser Ser Asp Glu Ala Gly Leu Leu Lys Met Val
1315 1320 1325

Asp Ala Ala Val His Ala Asp Ser Gly Thr Thr Leu Gln Leu Thr Ser
1330 1335 1340

Cys Leu Asn Gly Ser Gly Pro Val Pro Ala Pro Pro Pro Thr Pro Gly
1345 1350 1355 1360

Asn His Glu Arg Gly Ala Ala
1365

CLAIMS

What I claim is:

1. An isolated mammalian nucleic acid molecule encoding a receptor protein tyrosine kinase expressed in primitive hematopoietic cells and not expressed in mature hematopoietic cells.

2. A nucleic acid molecule according to claim 1 wherein the nucleic acid molecule is DNA.

3. A nucleic acid molecule according to claim 2 wherein the nucleic acid molecule is cDNA.

4. A nucleic acid molecule according to claim 1 wherein the nucleic acid molecule is RNA.

5. A nucleic acid molecule according to claim 1 that is a mouse nucleic acid molecule.

6. A nucleic acid molecule according to claim 5 that is flk-2 having the sequence shown in Figure 1a.

7. A nucleic acid molecule according to claim 1 that is a human nucleic acid molecule.

8. A nucleic acid molecule according to claim 7 that is DNA.

9. A nucleic acid molecule according to claim 7 that is flk-2 comprising the sequence shown in Figure 1b or 1c.

10. An isolated acid nucleic molecule that is flk-2 comprising the sequence shown in Figure 1a, 1b, or 1c.

11. A nucleic acid molecule according to claim 10 comprising the sequence shown in Figure 1b, or 1c.

12. A nucleic acid molecule according to claim 10 wherein the nucleic acid molecule is DNA.

13. A nucleic acid molecule according to claim 10 that has the corresponding sequence of RNA.

14. An isolated nucleic molecule that is flk-1 having the sequence shown in Figure 2.

15. A nucleic acid molecule according to claim 14 wherein the nucleic acid molecule is DNA.

16. A nucleic acid molecule according to claim 14 wherein the nucleic acid molecule is cDNA.

17. A nucleic acid molecule according to claim 14 that has the corresponding sequence of RNA.

18. A vector comprising a mammalian nucleic acid molecule encoding a receptor protein tyrosine kinase expressed in primitive hematopoietic cells and not expressed in mature hematopoietic cells.

19. A vector comprising flk-1 having the nucleic acid sequence of Figure 2.

20. A vector comprising flk-2 having the nucleic acid sequence of Figure 1a, 1b, or 1c.

21. A vector according to claim 18 wherein the vector is capable of being cloned in a host.

22. A vector according to claim 19 wherein the vector is capable of being cloned in a host.

23. A vector according to claim 20 wherein the vector is capable of being cloned in a host.

24. A vector according to claim 21 wherein the host is a

prokaryotic host.

25. A vector according to claim 22 wherein the host is a prokaryotic host.

26. A vector according to claim 23 wherein the host is a prokaryotic host.

27. A vector according to claim 18 that is capable of expressing the nucleic acid molecule in a host.

28. A vector according to claim 19 that is capable of expressing flk-1 in a host.

29. A vector according to claim 20 that is capable of expressing flk-2 in a host.

30. A vector according to claim 27 wherein the host is a prokaryotic host.

31. A vector according to claim 28 wherein the host is a prokaryotic host.

32. A vector according to claim 29 wherein the host is a prokaryotic host.

33. A vector according to claim 27 wherein the host is a eucaryotic host.

34. A vector according to claim 28 wherein the host is a eucaryotic host.

35. A vector according to claim 29 wherein the host is a eucaryotic host.

36. An isolated protein tyrosine kinase expressed in primitive hematopoietic cells and not expressed in mature hematopoietic cells.

37. The protein tyrosine kinase according to claim 36 that is flk-2 having the sequence shown in Figure 1a, 1b, or 1c.

38. The protein tyrosine kinase according to claim 36 that is human flk-2.

39. The protein tyrosine kinase according to claim 38 that is flk-2 having the sequence shown in Figure 1b or Figure 1c.

40. An isolated protein tyrosine kinase that is flk-1 having the sequence shown in Figure 2.

41. A ligand that binds to a receptor protein tyrosine kinase expressed in primitive mammalian hematopoietic cells and not expressed in mature hematopoietic cells, wherein the ligand stimulates the proliferation and/or differentiation of the primitive hematopoietic cells.

42. A ligand that binds to the receptor protein tyrosine kinase having the amino acid sequence of flk-1 shown in Figure 2, wherein the ligand stimulates the proliferation and/or differentiation of cells that express flk-1.

43. A ligand that binds to the receptor protein tyrosine kinase having the amino acid sequence of flk-2 shown in Figure 1a, 1b, or 1c, wherein the ligand stimulates the proliferation and/or differentiation of cells that express flk-2.

44. A nucleic acid molecule encoding a ligand that binds to a receptor protein tyrosine kinase expressed in primitive mammalian hematopoietic cells and not expressed in mature hematopoietic cells, wherein the ligand stimulates the proliferation and/or differentiation of the primitive hematopoietic cells.

45. A nucleic acid molecule encoding a ligand that binds to the receptor protein tyrosine kinase having the amino

acid sequence of flk-1 shown in Figure 2, wherein the ligand stimulates the proliferation and/or differentiation of cells that express flk-1.

46. A nucleic acid molecule encoding a ligand that binds to the receptor protein tyrosine kinase having the amino acid sequence of flk-2 shown in Figure 1a, 1b, or 1c wherein the ligand stimulates the proliferation and/or differentiation of cells that express flk-2.

47. A nucleic acid molecule according to claim 44 wherein the nucleic acid molecule is DNA.

48. A nucleic acid molecule according to claim 44 wherein the nucleic acid molecule is cDNA.

49. A nucleic acid molecule according to claim 44 wherein the nucleic acid molecule is RNA.

50. A nucleic acid molecule according to claim 45 wherein the nucleic acid molecule is DNA.

51. A nucleic acid molecule according to claim 45 wherein the nucleic acid molecule is cDNA.

52. A nucleic acid molecule according to claim 45 wherein the nucleic acid molecule is RNA.

53. A nucleic acid molecule according to claim 46 wherein the nucleic acid molecule is DNA.

54. A nucleic acid molecule according to claim 46 wherein the nucleic acid molecule is cDNA.

55. A nucleic acid molecule according to claim 46 wherein the nucleic acid molecule is RNA.

56. A method of stimulating the proliferation and/or differentiation of primitive mammalian hematopoietic stem

cells comprising contacting the stem cells with a ligand that binds to a receptor protein tyrosine kinase expressed in primitive mammalian hematopoietic cells and not expressed in mature hematopoietic cells.

57. A method of stimulating the proliferation and/or differentiation of primitive mammalian hematopoietic stem cells comprising contacting the stem cells with a ligand that binds to the receptor protein tyrosine kinase having the nucleic acid sequence of flk-1 shown in Figure 2.

58. A method of stimulating the proliferation and/or differentiation of primitive mammalian hematopoietic stem cells comprising contacting the stem cells with a ligand that binds to the receptor protein tyrosine kinase having the nucleic acid sequence of flk-2 shown in Figure 1a, 1b, or 1c.

59. A method according to claim 56 wherein the stimulation occurs in vitro.

60. A method according to claim 57 wherein the stimulation occurs in vitro.

61. A method according to claim 58 wherein the stimulation occurs in vitro.

62. A method according to claim 56 wherein the stimulation occurs in vivo.

63. A method according to claim 57 wherein the stimulation occurs in vivo.

64. A method according to claim 58 wherein the stimulation occurs in vivo.

65. Murine cell line 2018 having ATCC accession number ATCC CRL 10907.

FIG. 1a.1

```
GCGGCCTGGC TACCGCGCGC TCCGGAGGCC ATG CGG GCG TTG GCG CAG CGC AGC
                                 Met Arg Ala Leu Ala Gln Arg Ser
                                  1               5

GAC CGG CGG CTG CTG CTG CTT GTT GTT TTG TCA GTA ATG ATT CTT GAG
Asp Arg Arg Leu Leu Leu Leu Val Val Leu Ser Val Met Ile Leu Glu
     10                  15                  20

ACC GTT ACA AAC CAA GAC CTG CCT GTG ATC AAG TGT GTT TTA ATC AGT
Thr Val Thr Asn Gln Asp Leu Pro Val Ile Lys Cys Val Leu Ile Ser
 25                  30                  35                  40

CAT GAG AAC AAT GGC TCA TCA GCG GGA AAG CCA TCA TCG TAC CGA ATG
His Glu Asn Asn Gly Ser Ser Ala Gly Lys Pro Ser Ser Tyr Arg Met
                 45                  50                  55

GTG CGA GGA TCC CCA GAA GAC CTC CAG TGT ACC CCG AGG CGC CAG AGT
Val Arg Gly Ser Pro Glu Asp Leu Gln Cys Thr Pro Arg Arg Gln Ser
             60                  65                  70

GAA GGG ACG GTA TAT GAA GCG GCC ACC GTG GAG GTG GCC GAG TCT GGG
Glu Gly Thr Val Tyr Glu Ala Ala Thr Val Glu Val Ala Glu Ser Gly
         75                  80                  85

TCC ATC ACC CTG CAA GTG CAG CTC GCC ACC CCA GGG GAC CTT TCC TGC
Ser Ile Thr Leu Gln Val Gln Leu Ala Thr Pro Gly Asp Leu Ser Cys
     90                  95                 100

CTC TGG GTC TTT AAG CAC AGC TCC CTG GGC TGC CAG CCG CAC TTT GAT
Leu Trp Val Phe Lys His Ser Ser Leu Gly Cys Gln Pro His Phe Asp
105                 110                 115                 120

TTA CAA AAC AGA GGA ATC GTT TCC ATG GCC ATC TTG AAC GTG ACA GAG
Leu Gln Asn Arg Gly Ile Val Ser Met Ala Ile Leu Asn Val Thr Glu
                125                 130                 135

ACC CAG GCA GGA GAA TAC CTA CTC CAT ATT CAG AGC GAA CGC GCC AAC
Thr Gln Ala Gly Glu Tyr Leu Leu His Ile Gln Ser Glu Arg Ala Asn
            140                 145                 150

TAC ACA GTA CTG TTC ACA GTG AAT GTA AGA GAT ACA CAG CTG TAT GTG
Tyr Thr Val Leu Phe Thr Val Asn Val Arg Asp Thr Gln Leu Tyr Val
        155                 160                 165

CTA AGG AGA CCT TAC TTT AGG AAG ATG GAA AAC CAG GAT GCA CTG CTC
Leu Arg Arg Pro Tyr Phe Arg Lys Met Glu Asn Gln Asp Ala Leu Leu
    170                 175                 180

TGC ATC TCC GAG GGT GTT CCG GAG CCC ACT GTG GAG TGG GTG CTC TGC
Cys Ile Ser Glu Gly Val Pro Glu Pro Thr Val Glu Trp Val Leu Cys
185                 190                 195                 200

AGC TCC CAC AGG GAA AGC TGT AAA GAA GAA GGC CCT GCT GTT GTC AGA
Ser Ser His Arg Glu Ser Cys Lys Glu Glu Gly Pro Ala Val Val Arg
                205                 210                 215
```

FIG. 1a.1

```
AAG GAG GAA AAG GTA CTT CAT GAG TTG TTC GGA ACA GAC ATC AGA TGC
Lys Glu Glu Lys Val Leu His Glu Leu Phe Gly Thr Asp Ile Arg Cys
            220                 225                 230

TGT GCT AGA AAT GCA CTG GGC CGC GAA TGC ACC AAG CTG TTC ACC ATA
Cys Ala Arg Asn Ala Leu Gly Arg Glu Cys Thr Lys Leu Phe Thr Ile
        235                 240                 245

GAT CTA AAC CAG GCT CCT CAG AGC ACA CTG CCC CAG TTA TTC CTG AAA
Asp Leu Asn Gln Ala Pro Gln Ser Thr Leu Pro Gln Leu Phe Leu Lys
    250                 255                 260

GTG GGG GAA CCC TTG TGG ATC AGG TGT AAG GCC ATC CAT GTG AAC CAT
Val Gly Glu Pro Leu Trp Ile Arg Cys Lys Ala Ile His Val Asn His
265                 270                 275                 280

GGA TTC GGG CTC ACC TGG GAG CTG GAA GAC AAA GCC CTG GAG GAG GGC
Gly Phe Gly Leu Thr Trp Glu Leu Glu Asp Lys Ala Leu Glu Glu Gly
                285                 290                 295

AGC TAC TTT GAG ATG AGT ACC TAC TCC ACA AAC AGG ACC ATG ATT CGG
Ser Tyr Phe Glu Met Ser Thr Tyr Ser Thr Asn Arg Thr Met Ile Arg
            300                 305                 310

ATT CTC TTG GCC TTT GTG TCT TCC GTG GGA AGG AAC GAC ACC GGA TAT
Ile Leu Leu Ala Phe Val Ser Ser Val Gly Arg Asn Asp Thr Gly Tyr
        315                 320                 325

TAC ACC TGC TCT TCC TCA AAG CAC CCC AGC CAG TCA GCG TTG GTG ACC
Tyr Thr Cys Ser Ser Ser Lys His Pro Ser Gln Ser Ala Leu Val Thr
    330                 335                 340

ATC CTA GAA AAA GGG TTT ATA AAC GCT ACC AGC TCG CAA GAA GAG TAT
Ile Leu Glu Lys Gly Phe Ile Asn Ala Thr Ser Ser Gln Glu Glu Tyr
345                 350                 355                 360

GAA ATT GAC CCG TAC GAA AAG TTC TGC TTC TCA GTC AGG TTT AAA GCG
Glu Ile Asp Pro Tyr Glu Lys Phe Cys Phe Ser Val Arg Phe Lys Ala
                365                 370                 375

TAC CCA CGA ATC CGA TGC ACG TGG ATC TTC TCT CAA GCC TCA TTT CCT
Tyr Pro Arg Ile Arg Cys Thr Trp Ile Phe Ser Gln Ala Ser Phe Pro
            380                 385                 390

TGT GAA CAG AGA GGC CTG GAG GAT GGG TAC AGC ATA TCT AAA TTT TGC
Cys Glu Gln Arg Gly Leu Glu Asp Gly Tyr Ser Ile Ser Lys Phe Cys
        395                 400                 405

GAT CAT AAG AAC AAG CCA GGA GAG TAC ATA TTC TAT GCA GAA AAT GAT
Asp His Lys Asn Lys Pro Gly Glu Tyr Ile Phe Tyr Ala Glu Asn Asp
    410                 415                 420

GAC GCC CAG TTC ACC AAA ATG TTC ACG CTG AAT ATA AGA AAG AAA CCT
Asp Ala Gln Phe Thr Lys Met Phe Thr Leu Asn Ile Arg Lys Lys Pro
425                 430                 435                 440
```

FIG. 1a.2

CAA	GTG	CTA	GCA	AAT	GCC	TCA	GCC	AGC	CAG	GCG	TCC	TGT	TCC	TCT	GAT
Gln	Val	Leu	Ala	Asn	Ala	Ser	Ala	Ser	Gln	Ala	Ser	Cys	Ser	Ser	Asp
				445					450					455	
GGC	TAC	CCG	CTA	CCC	TCT	TGG	ACC	TGG	AAG	AAG	TGT	TCG	GAC	AAA	TCT
Gly	Tyr	Pro	Leu	Pro	Ser	Trp	Thr	Trp	Lys	Lys	Cys	Ser	Asp	Lys	Ser
			460					465					470		
CCC	AAT	TGC	ACG	GAG	GAA	ATC	CCA	GAA	GGA	GTT	TGG	AAT	AAA	AAG	GCT
Pro	Asn	Cys	Thr	Glu	Glu	Ile	Pro	Glu	Gly	Val	Trp	Asn	Lys	Lys	Ala
		475					480					485			
AAC	AGA	AAA	GTG	TTT	GGC	CAG	TGG	GTG	TCG	AGC	AGT	ACT	CTA	AAT	ATG
Asn	Arg	Lys	Val	Phe	Gly	Gln	Trp	Val	Ser	Ser	Ser	Thr	Leu	Asn	Met
	490					495					500				
AGT	GAG	GCC	GGG	AAA	GGG	CTT	CTG	GTC	AAA	TGC	TGT	GCG	TAC	AAT	TCT
Ser	Glu	Ala	Gly	Lys	Gly	Leu	Leu	Val	Lys	Cys	Cys	Ala	Tyr	Asn	Ser
505					510					515					520
ATG	GGC	ACG	TCT	TGC	GAA	ACC	ATC	TTT	TTA	AAC	TCA	CCA	GGC	CCC	TTC
Met	Gly	Thr	Ser	Cys	Glu	Thr	Ile	Phe	Leu	Asn	Ser	Pro	Gly	Pro	Phe
				525					530					535	
CCT	TTC	ATC	CAA	GAC	AAC	ATC	TCC	TTC	TAT	GCG	ACC	ATT	GGG	CTC	TGT
Pro	Phe	Ile	Gln	Asp	Asn	Ile	Ser	Phe	Tyr	Ala	Thr	Ile	Gly	Leu	Cys
			540					545					550		
CTC	CCC	TTC	ATT	GTT	GTT	CTC	ATT	GTG	TTG	ATC	TGC	CAC	AAA	TAC	AAA
Leu	Pro	Phe	Ile	Val	Val	Leu	Ile	Val	Leu	Ile	Cys	His	Lys	Tyr	Lys
		555					560					565			
AAG	CAA	TTT	AGG	TAC	GAG	AGT	CAG	CTG	CAG	ATG	ATC	CAG	GTG	ACT	GGC
Lys	Gln	Phe	Arg	Tyr	Glu	Ser	Gln	Leu	Gln	Met	Ile	Gln	Val	Thr	Gly
	570					575					580				
CCC	CTG	GAT	AAC	GAG	TAC	TTC	TAC	GTT	GAC	TTC	AGG	GAC	TAT	GAA	TAT
Pro	Leu	Asp	Asn	Glu	Tyr	Phe	Tyr	Val	Asp	Phe	Arg	Asp	Tyr	Glu	Tyr
585					590					595					600
GAC	CTT	AAG	TGG	GAG	TTC	CCG	AGA	GAG	AAC	TTA	GAG	TTT	GGG	AAG	GTC
Asp	Leu	Lys	Trp	Glu	Phe	Pro	Arg	Glu	Asn	Leu	Glu	Phe	Gly	Lys	Val
				605					610					615	
CTG	GGG	TCT	GGC	GCT	TTC	GGG	AGG	GTG	ATG	AAC	GCC	ACG	GCC	TAT	GGC
Leu	Gly	Ser	Gly	Ala	Phe	Gly	Arg	Val	Met	Asn	Ala	Thr	Ala	Tyr	Gly
			620					625					630		
ATT	AGT	AAA	ACG	GGA	GTC	TCA	ATT	CAG	GTG	GCG	GTG	AAG	ATG	CTA	AAA
Ile	Ser	Lys	Thr	Gly	Val	Ser	Ile	Gln	Val	Ala	Val	Lys	Met	Leu	Lys
		635					640					645			
GAG	AAA	GCT	GAC	AGC	TGT	GAA	AAA	GAA	GCT	CTC	ATG	TCG	GAG	CTC	AAA
Glu	Lys	Ala	Asp	Ser	Cys	Glu	Lys	Glu	Ala	Leu	Met	Ser	Glu	Leu	Lys
	650					655					660				

FIG. 1a.2

```
ATG ATG ACC CAC CTG GGA CAC CAT GAC AAC ATC GTG AAT CTG CTG GGG
Met Met Thr His Leu Gly His His Asp Asn Ile Val Asn Leu Leu Gly
665                 670                 675                 680

GCA TGC ACA CTG TCA GGG CCA GTG TAC TTG ATT TTT GAA TAT TGT TGC
Ala Cys Thr Leu Ser Gly Pro Val Tyr Leu Ile Phe Glu Tyr Cys Cys
                685                 690                 695

TAT GGT GAC CTC CTC AAC TAC CTA AGA AGT AAA AGA GAG AAG TTT CAC
Tyr Gly Asp Leu Leu Asn Tyr Leu Arg Ser Lys Arg Glu Lys Phe His
            700                 705                 710

AGG ACA TGG ACA GAG ATT TTT AAG GAA CAT AAT TTC AGT TCT TAC CCT
Arg Thr Trp Thr Glu Ile Phe Lys Glu His Asn Phe Ser Ser Tyr Pro
        715                 720                 725

ACT TTC CAG GCA CAT TCA AAT TCC AGC ATG CCT GGT TCA CGA GAA GTT
Thr Phe Gln Ala His Ser Asn Ser Ser Met Pro Gly Ser Arg Glu Val
    730                 735                 740

CAG TTA CAC CCG CCC TTG GAT CAG CTC TCA GGG TTC AAT GGG AAT TCA
Gln Leu His Pro Pro Leu Asp Gln Leu Ser Gly Phe Asn Gly Asn Ser
745                 750                 755                 760

ATT CAT TCT GAA GAT GAG ATT GAA TAT GAA AAC CAG AAG AGG CTG GCA
Ile His Ser Glu Asp Glu Ile Glu Tyr Glu Asn Gln Lys Arg Leu Ala
                765                 770                 775

GAA GAA GAG GAG GAA GAT TTG AAC GTG CTG ACG TTT GAA GAC CTC CTT
Glu Glu Glu Glu Glu Asp Leu Asn Val Leu Thr Phe Glu Asp Leu Leu
            780                 785                 790

TGC TTT GCG TAC CAA GTG GCC AAA GGC ATG GAA TTC CTG GAG TTC AAG
Cys Phe Ala Tyr Gln Val Ala Lys Gly Met Glu Phe Leu Glu Phe Lys
        795                 800                 805

TCG TGT GTC CAC AGA GAC CTG GCA GCC AGG AAT GTG TTG GTC ACC CAC
Ser Cys Val His Arg Asp Leu Ala Ala Arg Asn Val Leu Val Thr His
    810                 815                 820

GGG AAG GTG GTG AAG ATC TGT GAC TTT GGA CTG GCC CGA GAC ATC CTG
Gly Lys Val Val Lys Ile Cys Asp Phe Gly Leu Ala Arg Asp Ile Leu
825                 830                 835                 840

AGC GAC TCC AGC TAC GTC GTC AGG GGC AAC GCA CGG CTG CCG GTG AAG
Ser Asp Ser Ser Tyr Val Val Arg Gly Asn Ala Arg Leu Pro Val Lys
                845                 850                 855

TGG ATG GCA CCC GAG AGC TTA TTT GAA GGG ATC TAC ACA ATC AAG AGT
Trp Met Ala Pro Glu Ser Leu Phe Glu Gly Ile Tyr Thr Ile Lys Ser
            860                 865                 870

GAC GTC TGG TCC TAC GGC ATC CTT CTC TGG GAG ATA TTT TCA CTG GGT
Asp Val Trp Ser Tyr Gly Ile Leu Leu Trp Glu Ile Phe Ser Leu Gly
        875                 880                 885
```

FIG. 1a.3

```
GTG AAC CCT TAC CCT GGC ATT CCT GTC GAC GCT AAC TTC TAT AAA CTG
Val Asn Pro Tyr Pro Gly Ile Pro Val Asp Ala Asn Phe Tyr Lys Leu
    890                 895                 900

ATT CAG AGT GGA TTT AAA ATG GAG CAG CCA TTC TAT GCC ACA GAA GGG
Ile Gln Ser Gly Phe Lys Met Glu Gln Pro Phe Tyr Ala Thr Glu Gly
905                 910                 915                 920

ATA TAC TTT GTA ATG CAA TCC TGC TGG GCT TTT GAC TCA AGG AAG CGG
Ile Tyr Phe Val Met Gln Ser Cys Trp Ala Phe Asp Ser Arg Lys Arg
                925                 930                 935

CCA TCC TTC CCC AAC CTG ACT TCA TTT TTA GGA TGT CAG CTG GCA GAG
Pro Ser Phe Pro Asn Leu Thr Ser Phe Leu Gly Cys Gln Leu Ala Glu
            940                 945                 950

GCA GAA GAA GCA TGT ATC AGA ACA TCC ATC CAT CTA CCA AAA CAG GCG
Ala Glu Glu Ala Cys Ile Arg Thr Ser Ile His Leu Pro Lys Gln Ala
        955                 960                 965

GCC CCT CAG CAG AGA GGC GGG CTC AGA GCC CAG TCG CCA CAG CGC CAG
Ala Pro Gln Gln Arg Gly Gly Leu Arg Ala Gln Ser Pro Gln Arg Gln
    970                 975                 980

GTG AAG ATT CAC AGA GAA AGA AGT TAGCGAGGAG GCCTTGGACC CCGCCACCCT
Val Lys Ile His Arg Glu Arg Ser
985                 990

AGCAGGCTGT AGACCGCAGA GCCAAGATTA GCCTCGCCTC TGAGGAAGCG CCCTACAGCG

CGTTGCTTCG CTGGACTTTT CTCTAGATGC TGTCTGCCAT TACTCCAAAG TGACTTCTAT

AAAATCAAAC CTCTCCTCGC ACAGGCGGGA GAGCCAATAA TGAGACTTGT TGGTGAGCCC

GCCTACCCTG GGGGCCTTTC CACGAGCTTG AGGGGAAAGC CATGTATCTG AAATATAGTA

TATTCTTGTA AATACGTGAA ACAAACCAAA CCCGTTTTTT GCTAAGGGAA AGCTAAATAT

GATTTTTAAA AATCTATGTT TTAAAATACT ATGTAACTTT TTCATCTATT TAGTGATATA

TTTTATGGAT GGAAATAAAC TTTCTACTGT AAAAAAAAAA AAAAAAAAAA AAAAAAA
```

FIG. 1b.

```
AAC AAT GAT TCA TCA GTG GGG AAG TCA TCA TCA TAT CCC ATG GTA TCA
Asn Asn Asp Ser Ser Val Gly Lys Ser Ser Ser Tyr Pro Met Val Ser
  1             5                  10                  15

GAA TCC CCG GAA GAC CTC GGG TGT GCG TTG AGA CCC CAG AGC TCA GGG
Glu Ser Pro Glu Asp Leu Gly Cys Ala Leu Arg Pro Gln Ser Ser Gly
             20                  25                  30

ACA GTG TAC GAA GCT GCC GCT GTG GAA GTG GAT GTA TCT GCT TCC ATC
Thr Val Tyr Glu Ala Ala Ala Val Glu Val Asp Val Ser Ala Ser Ile
         35                  40                  45

ACA CTG CAA GTG CTG GTC GAT GCC CCA GGG AAC ATT TCC TGT CTC TGG
Thr Leu Gln Val Leu Val Asp Ala Pro Gly Asn Ile Ser Cys Leu Trp
     50                  55                  60

GTC TTT AAG CAC AGC TCC CTG AAT TGC CAG CCA CAT TTT GAT TTA CAA
Val Phe Lys His Ser Ser Leu Asn Cys Gln Pro His Phe Asp Leu Gln
 65                  70                  75                  80

AAC AGA GGA GTT GTT TCC ATG GTC ATT TTG AAA ATG ACA GAA ACC CAA
Asn Arg Gly Val Val Ser Met Val Ile Leu Lys Met Thr Glu Thr Gln
                 85                  90                  95

GCT GGA GAA TAC CTA CTT TTT ATT CAG AGT GAA GCT ACC AAT TA
Ala Gly Glu Tyr Leu Leu Phe Ile Gln Ser Glu Ala Thr Asn
            100                 105                 110
```

FIG. 1c.

```
GAT CAA ATC TCA GGC TTC ATG GAA TTC ATT CAC TCT GAA GAT GAA ATT
Asp Gln Ile Ser Gly Phe Met Glu Phe Ile His Ser Glu Asp Glu Ile
 1               5                  10                  15

GAA TAT GAA AAC CAA AAA AAG AGG CTG GAA GAA GAG GAG GAC TTG AAT
Glu Tyr Glu Asn Gln Lys Lys Arg Leu Glu Glu Glu Glu Asp Leu Asn
            20                  25                  30

GTG CTT ACA TTT GAA GAT CTT CTT TGC TTT GCA TAT CAA GTT GCC AAA
Val Leu Thr Phe Glu Asp Leu Leu Cys Phe Ala Tyr Gln Val Ala Lys
        35                  40                  45

GGA ATG GAA TTT AAG TCG TGT GTT CAC AGA GAC CTG GCC GCC AGG AAC
Gly Met Glu Phe Lys Ser Cys Val His Arg Asp Leu Ala Ala Arg Asn
    50                  55                  60

GTG CTT GTC ACC CAC GGG AAA GTG GTG AAG ATA TGT GAC TTT GGA TTG
Val Leu Val Thr His Gly Lys Val Val Lys Ile Cys Asp Phe Gly Leu
65                  70                  75                  80

GCT CGA GAT ATC ATG AGT GAT TCC GGC TAT GTT GTC AGG CAA
Ala Arg Asp Ile Met Ser Asp Ser Gly Tyr Val Val Arg Gln
                85                  90

TC
```

FIG. 2.

```
CTGTGTCCCG CAGCCGGATA ACCTGGCTGA CCCGATTCCG CGGACACCCG TGCAGCCGCG

GCTGGAGCCA GGGCGCCGGT GCCCGCGCTC TCCCCGGTCT TGCGCTGCGG GGGCCGATAC

CGCCTCTGTG ACTTCTTTGC GGGCCAGGGA CGGAGAAGGA GTCTGTGCCT GAGAAACTGG

GCTCTGTGCC CAGGCGCGAG GTGCAGG ATG GAG AGC AAG GGC CTG CTA GCT
                              Met Glu Ser Lys Gly Leu Leu Ala
                               1               5

GTC GCT CTG TGG TTC TGC GTG GAG ACC CGA GCC GCC TCT GTG GGT TTG
Val Ala Leu Trp Phe Cys Val Glu Thr Arg Ala Ala Ser Val Gly Leu
     10                  15                  20

CCT GGC GAT TTT CTC CAT CCC CCC AAG CTC AGC ACA CAG AAA GAC ATA
Pro Gly Asp Phe Leu His Pro Pro Lys Leu Ser Thr Gln Lys Asp Ile
 25                  30                  35                  40

CTG ACA ATT TTG GCA AAT ACA ACC CTT CAG ATT ACT TGC AGG GGA CAG
Leu Thr Ile Leu Ala Asn Thr Thr Leu Gln Ile Thr Cys Arg Gly Gln
                 45                  50                  55

CGG GAC CTG GAC TGG CTT TGG CCC AAT GCT CAG CGT GAT TCT GAG GAA
Arg Asp Leu Asp Trp Leu Trp Pro Asn Ala Gln Arg Asp Ser Glu Glu
             60                  65                  70

AGG GTA TTG GTG ACT GAA TGC GGC GGT GGT GAC AGT ATC TTC TGC AAA
Arg Val Leu Val Thr Glu Cys Gly Gly Gly Asp Ser Ile Phe Cys Lys
         75                  80                  85

ACA CTC ACC ATT CCC AGG GTG GTT GGA AAT GAT ACT GGA GCC TAC AAG
Thr Leu Thr Ile Pro Arg Val Val Gly Asn Asp Thr Gly Ala Tyr Lys
     90                  95                 100

TGC TCG TAC CGG GAC GTC GAC ATA GCC TCC ACT GTT TAT GTC TAT GTT
Cys Ser Tyr Arg Asp Val Asp Ile Ala Ser Thr Val Tyr Val Tyr Val
105                 110                 115                 120

CGA GAT TAC AGA TCA CCA TTC ATC GCC TCT GTC AGT GAC CAG CAT GGC
Arg Asp Tyr Arg Ser Pro Phe Ile Ala Ser Val Ser Asp Gln His Gly
                125                 130                 135

ATC GTG TAC ATC ACC GAG AAC AAG AAC AAA ACT GTG GTG ATC CCC TGC
Ile Val Tyr Ile Thr Glu Asn Lys Asn Lys Thr Val Val Ile Pro Cys
            140                 145                 150

CGA GGG TCG ATT TCA AAC CTC AAT GTG TCT CTT TGC GCT AGG TAT CCA
Arg Gly Ser Ile Ser Asn Leu Asn Val Ser Leu Cys Ala Arg Tyr Pro
        155                 160                 165

GAA AAG AGA TTT GTT CCG GAT GGA AAC AGA ATT TCC TGG GAC AGC GAG
Glu Lys Arg Phe Val Pro Asp Gly Asn Arg Ile Ser Trp Asp Ser Glu
    170                 175                 180
```

FIG. 2.

ATA	GGC	TTT	ACT	CTC	CCC	AGT	TAC	ATG	ATC	AGC	TAT	GCC	GGC	ATG	GTC
Ile	Gly	Phe	Thr	Leu	Pro	Ser	Tyr	Met	Ile	Ser	Tyr	Ala	Gly	Met	Val
185					190					195					200
TTC	TGT	GAG	GCA	AAG	ATC	AAT	GAT	GAA	ACC	TAT	CAG	TCT	ATC	ATG	TAC
Phe	Cys	Glu	Ala	Lys	Ile	Asn	Asp	Glu	Thr	Tyr	Gln	Ser	Ile	Met	Tyr
				205					210					215	
ATA	GTT	GTG	GTT	GTA	GGA	TAT	AGG	ATT	TAT	GAT	GTG	ATT	CTG	AGC	CCC
Ile	Val	Val	Val	Val	Gly	Tyr	Arg	Ile	Tyr	Asp	Val	Ile	Leu	Ser	Pro
			220					225					230		
CCG	CAT	GAA	ATT	GAG	CTA	TCT	GCC	GGA	GAA	AAA	CTT	GTC	TTA	AAT	TGT
Pro	His	Glu	Ile	Glu	Leu	Ser	Ala	Gly	Glu	Lys	Leu	Val	Leu	Asn	Cys
		235					240					245			
ACA	GCG	AGA	ACA	GAG	CTC	AAT	GTG	GGG	CTT	GAT	TTC	ACC	TGG	CAC	TCT
Thr	Ala	Arg	Thr	Glu	Leu	Asn	Val	Gly	Leu	Asp	Phe	Thr	Trp	His	Ser
	250					255					260				
CCA	CCT	TCA	AAG	TCT	CAT	CAT	AAG	AAG	ATT	GTA	AAC	CGG	GAT	GTG	AAA
Pro	Pro	Ser	Lys	Ser	His	His	Lys	Lys	Ile	Val	Asn	Arg	Asp	Val	Lys
265					270					275					280
CCC	TTT	CCT	GGG	ACT	GTG	GCG	AAG	ATG	TTT	TTG	AGC	ACC	TTG	ACA	ATA
Pro	Phe	Pro	Gly	Thr	Val	Ala	Lys	Met	Phe	Leu	Ser	Thr	Leu	Thr	Ile
				285					290					295	
GAA	AGT	GTG	ACC	AAG	AGT	GAC	CAA	GGG	GAA	TAC	ACC	TGT	GTA	GCG	TCC
Glu	Ser	Val	Thr	Lys	Ser	Asp	Gln	Gly	Glu	Tyr	Thr	Cys	Val	Ala	Ser
			300					305					310		
AGT	GGA	CGG	ATG	ATC	AAG	AGA	AAT	AGA	ACA	TTT	GTC	CGA	GTT	CAC	ACA
Ser	Gly	Arg	Met	Ile	Lys	Arg	Asn	Arg	Thr	Phe	Val	Arg	Val	His	Thr
		315					320					325			
AAG	CCT	TTT	ATT	GCT	TTC	GGT	AGT	GGG	ATG	AAA	TCT	TTG	GTG	GAA	GCC
Lys	Pro	Phe	Ile	Ala	Phe	Gly	Ser	Gly	Met	Lys	Ser	Leu	Val	Glu	Ala
	330					335					340				
ACA	GTG	GGC	AGT	CAA	GTC	CGA	ATC	CCT	GTG	AAG	TAT	CTC	AGT	TAC	CCA
Thr	Val	Gly	Ser	Gln	Val	Arg	Ile	Pro	Val	Lys	Tyr	Leu	Ser	Tyr	Pro
345					350					355					360
GCT	CCT	GAT	ATC	AAA	TGG	TAC	AGA	AAT	GGA	AGG	CCC	ATT	GAG	TCC	AAC
Ala	Pro	Asp	Ile	Lys	Trp	Tyr	Arg	Asn	Gly	Arg	Pro	Ile	Glu	Ser	Asn
				365					370					375	
TAC	ACA	ATG	ATT	GTT	GGC	GAT	GAA	CTC	ACC	ATC	ATG	GAA	GTG	ACT	GAA
Tyr	Thr	Met	Ile	Val	Gly	Asp	Glu	Leu	Thr	Ile	Met	Glu	Val	Thr	Glu
			380					385					390		
AGA	GAT	GCA	GGA	AAC	TAC	ACG	GTC	ATC	CTC	ACC	AAC	CCC	ATT	TCA	ATG
Arg	Asp	Ala	Gly	Asn	Tyr	Thr	Val	Ile	Leu	Thr	Asn	Pro	Ile	Ser	Met
		395					400					405			

FIG. 2.1

GAG	AAA	CAG	AGC	CAC	ATG	GTC	TCT	CTG	GTT	GTG	AAT	GTC	CCA	CCC	CAG
Glu	Lys	Gln	Ser	His	Met	Val	Ser	Leu	Val	Val	Asn	Val	Pro	Pro	Gln
	410					415					420				
ATC	GGT	GAG	AAA	GCC	TTG	ATC	TCG	CCT	ATG	GAT	TCC	TAC	CAG	TAT	GGG
Ile	Gly	Glu	Lys	Ala	Leu	Ile	Ser	Pro	Met	Asp	Ser	Tyr	Gln	Tyr	Gly
425					430					435					440
ACC	ATG	CAG	ACA	TTG	ACA	TGC	ACA	GTC	TAC	GCC	AAC	CCT	CCC	CTG	CAC
Thr	Met	Gln	Thr	Leu	Thr	Cys	Thr	Val	Tyr	Ala	Asn	Pro	Pro	Leu	His
				445					450					455	
CAC	ATC	CAG	TGG	TAC	TGG	CAG	CTA	GAA	GAA	GCC	TGC	TCC	TAC	AGA	CCC
His	Ile	Gln	Trp	Tyr	Trp	Gln	Leu	Glu	Glu	Ala	Cys	Ser	Tyr	Arg	Pro
			460					465					470		
GGC	CAA	ACA	AGC	CCG	TAT	GCT	TGT	AAA	GAA	TGG	AGA	CAC	GTG	GAG	GAT
Gly	Gln	Thr	Ser	Pro	Tyr	Ala	Cys	Lys	Glu	Trp	Arg	His	Val	Glu	Asp
		475					480					485			
TTC	CAG	GGG	GGA	AAC	AAG	ATC	GAA	GTC	ACC	AAA	AAC	CAA	TAT	GCC	CTG
Phe	Gln	Gly	Gly	Asn	Lys	Ile	Glu	Val	Thr	Lys	Asn	Gln	Tyr	Ala	Leu
	490					495					500				
ATT	GAA	GGA	AAA	AAC	AAA	ACT	GTA	AGT	ACG	CTG	GTC	ATC	CAA	GCT	GCC
Ile	Glu	Gly	Lys	Asn	Lys	Thr	Val	Ser	Thr	Leu	Val	Ile	Gln	Ala	Ala
505					510					515					520
AAC	GTG	TCA	GCG	TTG	TAC	AAA	TGT	GAA	GCC	ATC	AAC	AAA	GCG	GGA	CGA
Asn	Val	Ser	Ala	Leu	Tyr	Lys	Cys	Glu	Ala	Ile	Asn	Lys	Ala	Gly	Arg
				525					530					535	
GGA	GAG	AGG	GTC	ATC	TCC	TTC	CAT	GTG	ATC	AGG	GGT	CCT	GAA	ATT	ACT
Gly	Glu	Arg	Val	Ile	Ser	Phe	His	Val	Ile	Arg	Gly	Pro	Glu	Ile	Thr
			540					545					550		
GTG	CAA	CCT	GCT	GCC	CAG	CCA	ACT	GAG	CAG	GAG	AGT	GTG	TCC	CTG	TTG
Val	Gln	Pro	Ala	Ala	Gln	Pro	Thr	Glu	Gln	Glu	Ser	Val	Ser	Leu	Leu
		555					560					565			
TGC	ACT	GCA	GAC	AGA	AAT	ACG	TTT	GAG	AAC	CTC	ACG	TGG	TAC	AAG	CTT
Cys	Thr	Ala	Asp	Arg	Asn	Thr	Phe	Glu	Asn	Leu	Thr	Trp	Tyr	Lys	Leu
	570					575					580				
GGC	TCA	CAG	GCA	ACA	TCG	GTC	CAC	ATG	GGC	GAA	TCA	CTC	ACA	CCA	GTT
Gly	Ser	Gln	Ala	Thr	Ser	Val	His	Met	Gly	Glu	Ser	Leu	Thr	Pro	Val
585					590					595					600
TGC	AAG	AAC	TTG	GAT	GCT	CTT	TGG	AAA	CTG	AAT	GGC	ACC	ATG	TTT	TCT
Cys	Lys	Asn	Leu	Asp	Ala	Leu	Trp	Lys	Leu	Asn	Gly	Thr	Met	Phe	Ser
				605					610					615	
AAC	AGC	ACA	AAT	GAC	ATC	TTG	ATT	GTG	GCA	TTT	CAG	AAT	GCC	TCT	CTG
Asn	Ser	Thr	Asn	Asp	Ile	Leu	Ile	Val	Ala	Phe	Gln	Asn	Ala	Ser	Leu
			620					625					630		

FIG. 2.1

```
CAG GAC CAA GGC GAC TAT GTT TGC TCT GCT CAA GAT AAG AAG ACC AAG
Gln Asp Gln Gly Asp Tyr Val Cys Ser Ala Gln Asp Lys Lys Thr Lys
        635                 640                 645

AAA AGA CAT TGC CTG GTC AAA CAG CTC ATC ATC CTA GAG CGC ATG GCA
Lys Arg His Cys Leu Val Lys Gln Leu Ile Ile Leu Glu Arg Met Ala
    650                 655                 660

CCC ATG ATC ACC GGA AAT CTG GAG AAT CAG ACA ACA ACC ATT GGC GAG
Pro Met Ile Thr Gly Asn Leu Glu Asn Gln Thr Thr Thr Ile Gly Glu
665                 670                 675                 680

ACC ATT GAA GTG ACT TGC CCA GCA TCT GGA AAT CCT ACC CCA CAC ATT
Thr Ile Glu Val Thr Cys Pro Ala Ser Gly Asn Pro Thr Pro His Ile
                685                 690                 695

ACA TGG TTC AAA GAC AAC GAG ACC CTG GTA GAA GAT TCA GGC ATT GTA
Thr Trp Phe Lys Asp Asn Glu Thr Leu Val Glu Asp Ser Gly Ile Val
            700                 705                 710

CTG AGA GAT GGG AAC CGG AAC CTG ACT ATC CGC AGG GTG AGG AAG GAG
Leu Arg Asp Gly Asn Arg Asn Leu Thr Ile Arg Arg Val Arg Lys Glu
        715                 720                 725

GAT GGA GGC CTC TAC ACC TGC CAG GCC TGC AAT GTC CTT GGC TGT GCA
Asp Gly Gly Leu Tyr Thr Cys Gln Ala Cys Asn Val Leu Gly Cys Ala
    730                 735                 740

AGA GCG GAG ACG CTC TTC ATA ATA GAA GGT GCC CAG GAA AAG ACC AAC
Arg Ala Glu Thr Leu Phe Ile Ile Glu Gly Ala Gln Glu Lys Thr Asn
745                 750                 755                 760

TTG GAA GTC ATT ATC CTC GTC GGC ACT GCA GTG ATT GCC ATG TTC TTC
Leu Glu Val Ile Ile Leu Val Gly Thr Ala Val Ile Ala Met Phe Phe
                765                 770                 775

TGG CTC CTT CTT GTC ATT CTC GTA CGG ACC GTT AAG CGG GCC AAT GAA
Trp Leu Leu Leu Val Ile Leu Val Arg Thr Val Lys Arg Ala Asn Glu
            780                 785                 790

GGG GAA CTG AAG ACA GGC TAC TTG TCT ATT GTC ATG GAT CCA GAT GAA
Gly Glu Leu Lys Thr Gly Tyr Leu Ser Ile Val Met Asp Pro Asp Glu
        795                 800                 805

TTG CCC TTG GAT GAG CGC TGT GAA CGC TTG CCT TAT GAT GCC AGC AAG
Leu Pro Leu Asp Glu Arg Cys Glu Arg Leu Pro Tyr Asp Ala Ser Lys
    810                 815                 820

TGG GAA TTC CCC AGG GAC CGG CTG AAA CTA GGA AAA CCT CTT GGC CGC
Trp Glu Phe Pro Arg Asp Arg Leu Lys Leu Gly Lys Pro Leu Gly Arg
825                 830                 835                 840

GGT GCC TTC GGC CAA GTG ATT GAG GCA GAC GCT TTT GGA ATT GAC AAG
Gly Ala Phe Gly Gln Val Ile Glu Ala Asp Ala Phe Gly Ile Asp Lys
                845                 850                 855
```

FIG. 2.2

```
ACA GCG ACT TGC AAA ACA GTA GCC GTC AAG ATG TTG AAA GAA GGA GCA
Thr Ala Thr Cys Lys Thr Val Ala Val Lys Met Leu Lys Glu Gly Ala
        860                 865                 870

ACA CAC AGC GAG CAT CGA GCC CTC ATG TCT GAA CTC AAG ATC CTC ATC
Thr His Ser Glu His Arg Ala Leu Met Ser Glu Leu Lys Ile Leu Ile
    875                 880                 885

CAC ATT GGT CAC CAT CTC AAT GTG GTG AAC CTC CTA GGC GCC TGC ACC
His Ile Gly His His Leu Asn Val Val Asn Leu Leu Gly Ala Cys Thr
890                 895                 900

AAG CCG GGA GGG CCT CTC ATG GTG ATT GTG GAA TTC TCG AAG TTT GGA
Lys Pro Gly Gly Pro Leu Met Val Ile Val Glu Phe Ser Lys Phe Gly
905                 910                 915                 920

AAC CTA TCA ACT TAC TTA CGG GGC AAG AGA AAT GAA TTT GTT CCC TAT
Asn Leu Ser Thr Tyr Leu Arg Gly Lys Arg Asn Glu Phe Val Pro Tyr
                925                 930                 935

AAG AGC AAA GGG GCA CGC TTC CGC CAG GGC AAG GAC TAC GTT GGG GAG
Lys Ser Lys Gly Ala Arg Phe Arg Gln Gly Lys Asp Tyr Val Gly Glu
            940                 945                 950

CTC TCC GTG GAT CTG AAA AGA CGC TTG GAC AGC ATC ACC AGC AGC CAG
Leu Ser Val Asp Leu Lys Arg Arg Leu Asp Ser Ile Thr Ser Ser Gln
        955                 960                 965

AGC TCT GCC AGC TCA GGC TTT GTT GAG GAG AAA TCG CTC AGT GAT GTA
Ser Ser Ala Ser Ser Gly Phe Val Glu Glu Lys Ser Leu Ser Asp Val
    970                 975                 980

GAG GAA GAA GAA GCT TCT GAA GAA CTG TAC AAG GAC TTC CTG ACC TTG
Glu Glu Glu Glu Ala Ser Glu Glu Leu Tyr Lys Asp Phe Leu Thr Leu
985                 990                 995                 1000

GAG CAT CTC ATC TGT TAC AGC TTC CAA GTG GCT AAG GGC ATG GAG TTC
Glu His Leu Ile Cys Tyr Ser Phe Gln Val Ala Lys Gly Met Glu Phe
                1005                1010                1015

TTG GCA TCA AGG AAG TGT ATC CAC AGG GAC CTG GCA GCA CGA AAC ATT
Leu Ala Ser Arg Lys Cys Ile His Arg Asp Leu Ala Ala Arg Asn Ile
            1020                1025                1030

CTC CTA TCG GAG AAG AAT GTG GTT AAG ATC TGT GAC TTC GGC TTG GCC
Leu Leu Ser Glu Lys Asn Val Val Lys Ile Cys Asp Phe Gly Leu Ala
        1035                1040                1045

CGG GAC ATT TAT AAA GAC CCG GAT TAT GTC AGA AAA GGA GAT GCC CGA
Arg Asp Ile Tyr Lys Asp Pro Asp Tyr Val Arg Lys Gly Asp Ala Arg
    1050                1055                1060

CTC CCT TTG AAG TGG ATG GCC CCG GAA ACC ATT TTT GAC AGA GTA TAC
Leu Pro Leu Lys Trp Met Ala Pro Glu Thr Ile Phe Asp Arg Val Tyr
1065                1070                1075                1080
```

FIG. 2.2

ACA ATT CAG AGC GAT GTG TGG TCT TTC GGT GTG TTG CTC TGG GAA ATA
Thr Ile Gln Ser Asp Val Trp Ser Phe Gly Val Leu Leu Trp Glu Ile
1085 1090 1095

TTT TCC TTA GGT GCC TCC CCA TAC CCT GGG GTC AAG ATT GAT GAA GAA
Phe Ser Leu Gly Ala Ser Pro Tyr Pro Gly Val Lys Ile Asp Glu Glu
1100 1105 1110

TTT TGT AGG AGA TTG AAA GAA GGA ACT AGA ATG CGG GCT CCT GAC TAC
Phe Cys Arg Arg Leu Lys Glu Gly Thr Arg Met Arg Ala Pro Asp Tyr
1115 1120 1125

ACT ACC CCA GAA ATG TAC CAG ACC ATG CTG GAC TGC TGG CAT GAG GAC
Thr Thr Pro Glu Met Tyr Gln Thr Met Leu Asp Cys Trp His Glu Asp
1130 1135 1140

CCC AAC CAG AGA CCC TCG TTT TCA GAG TTG GTG GAG CAT TTG GGA AAC
Pro Asn Gln Arg Pro Ser Phe Ser Glu Leu Val Glu His Leu Gly Asn
1145 1150 1155 1160

CTC CTG CAA GCA AAT GCG CAG CAG GAT GGC AAA GAC TAT ATT GTT CTT
Leu Leu Gln Ala Asn Ala Gln Gln Asp Gly Lys Asp Tyr Ile Val Leu
1165 1170 1175

CCA ATG TCA GAG ACA CTG AGC ATG GAA GAG GAT TCT GGA CTC TCC CTG
Pro Met Ser Glu Thr Leu Ser Met Glu Glu Asp Ser Gly Leu Ser Leu
1180 1185 1190

CCT ACC TCA CCT GTT TCC TGT ATG GAG GAA GAG GAA GTG TGC GAC CCC
Pro Thr Ser Pro Val Ser Cys Met Glu Glu Glu Glu Val Cys Asp Pro
1195 1200 1205

AAA TTC CAT TAT GAC AAC ACA GCA GGA ATC AGT CAT TAT CTC CAG AAC
Lys Phe His Tyr Asp Asn Thr Ala Gly Ile Ser His Tyr Leu Gln Asn
1210 1215 1220

AGT AAG CGA AAG AGC CGG CCA GTG AGT GTA AAA ACA TTT GAA GAT ATC
Ser Lys Arg Lys Ser Arg Pro Val Ser Val Lys Thr Phe Glu Asp Ile
1225 1230 1235 1240

CCA TTG GAG GAA CCA GAA GTA AAA GTG ATC CCA GAT GAC AGC CAG ACA
Pro Leu Glu Glu Pro Glu Val Lys Val Ile Pro Asp Asp Ser Gln Thr
1245 1250 1255

GAC AGT GGG ATG GTC CTT GCA TCA GAA GAG CTG AAA ACT CTG GAA GAC
Asp Ser Gly Met Val Leu Ala Ser Glu Glu Leu Lys Thr Leu Glu Asp
1260 1265 1270

AGG AAC AAA TTA TCT CCA TCT TTT GGT GGA ATG ATG CCC AGT AAA AGC
Arg Asn Lys Leu Ser Pro Ser Phe Gly Gly Met Met Pro Ser Lys Ser
1275 1280 1285

AGG GAG TCT GTG GCC TCG GAA GGC TCC AAC CAG ACC AGT GGC TAC CAG
Arg Glu Ser Val Ala Ser Glu Gly Ser Asn Gln Thr Ser Gly Tyr Gln
1290 1295 1300

FIG. 2.3

```
TCT GGG TAT CAC TCA GAT GAC ACA GAC ACC ACC GTG TAC TCC AGC GAC
Ser Gly Tyr His Ser Asp Asp Thr Asp Thr Thr Val Tyr Ser Ser Asp
1305                1310                1315                1320

GAG GCA GGA CTT TTA AAG ATG GTG GAT GCT GCA GTT CAC GCT GAC TCA
Glu Ala Gly Leu Leu Lys Met Val Asp Ala Ala Val His Ala Asp Ser
                1325                1330                1335

GGG ACC ACA CTG CAG CTC ACC TCC TGT TTA AAT GGA AGT GGT CCT GTC
Gly Thr Thr Leu Gln Leu Thr Ser Cys Leu Asn Gly Ser Gly Pro Val
            1340                1345                1350

CCG GCT CCG CCC CCA ACT CCT GGA AAT CAC GAG AGA GGT GCT GCT TAG
Pro Ala Pro Pro Pro Thr Pro Gly Asn His Glu Arg Gly Ala Ala
        1355                1360                1365

ATTTTCAAGT GTTGTTCTTT CCACCACCCG GAAGTAGCCA CATTTGATTT TCATTTTTGG

AGGAGGGACC TCAGACTGCA AGGAGCTTGT CCTCAGGGCA TTTCCAGAGA AGATGCCCAT

GACCCAAGAA TGTGTTGACT CTACTCTCTT TTCCATTCAT TTAAAAGTCC TATATAATGT

GCCCTGCTGT GGTCTCACTA CCAGTTAAAG CAAAAGACTT TCAAACACGT GGACTCTGTC

CTCCAAGAAG TGGCAACGGC ACCTCTGTGA AACTGGATCG AATGGGCAAT GCTTTGTGTG

TTGAGGATGG GTGAGATGTC CCAGGGCCGA GTCTGTCTAC CTTGGAGGCT TTGTGGAGGA

TGCGGCTATG AGCCAAGTGT TAAGTGTGGG ATGTGGACTG GGAGGAAGGA AGGCGCAAGC

CGTCCGGAGA GCGGTTGGAG CCTGCAGATG CATTGTGCTG GCTCTGGTGG AGGTGGGCTT

GTGGCCTGTC AGGAAACGCA AAGGCGGCCG GCAGGGTTTG GTTTTGGAAG GTTTGCGTGC

TCTTCACAGT CGGGTTACAG GCGAGTTCCC TGTGGCGTTT CCTACTCCTA ATGAGAGTTC

CTTCCGGACT CTTACGTGTC TCCTGGCCTG GCCCCAGGAA GGAAATGATG CAGCTTGCTC

CTTCCTCATC TCTCAGGCTG TGCCTTAATT CAGAACACCA AAAGAGAGGA ACGTCGGCAG

AGGCTCCTGA CGGGGCCGAA GAATTGTGAG AACAGAACAG AAACTCAGGG TTTCTGCTGG

GTGGAGACCC ACGTGGCGCC CTGGTGGCAG GTCTGAGGGT TCTCTGTCAA GTGGCGGTAA

AGGCTCAGGC TGGTGTTCTT CCTCTATCTC CACTCCTGTC AGGCCCCCAA GTCCTCAGTA

TTTTAGCTTT GTGGCTTCCT GATGGCAGAA AAATCTTAAT TGGTTGGTTT GCTCTCCAGA

TAATCACTAG CCAGATTTCG AAATTACTTT TTAGCCGAGG TTATGATAAC ATCTACTGTA

TCCTTTAGAA TTTTAACCTA TAAAACTATG TCTACTGGTT TCTGCCTGTG TGCTTATGTT

AAAAAAAAAA AAAAA
```

FIGURE 3

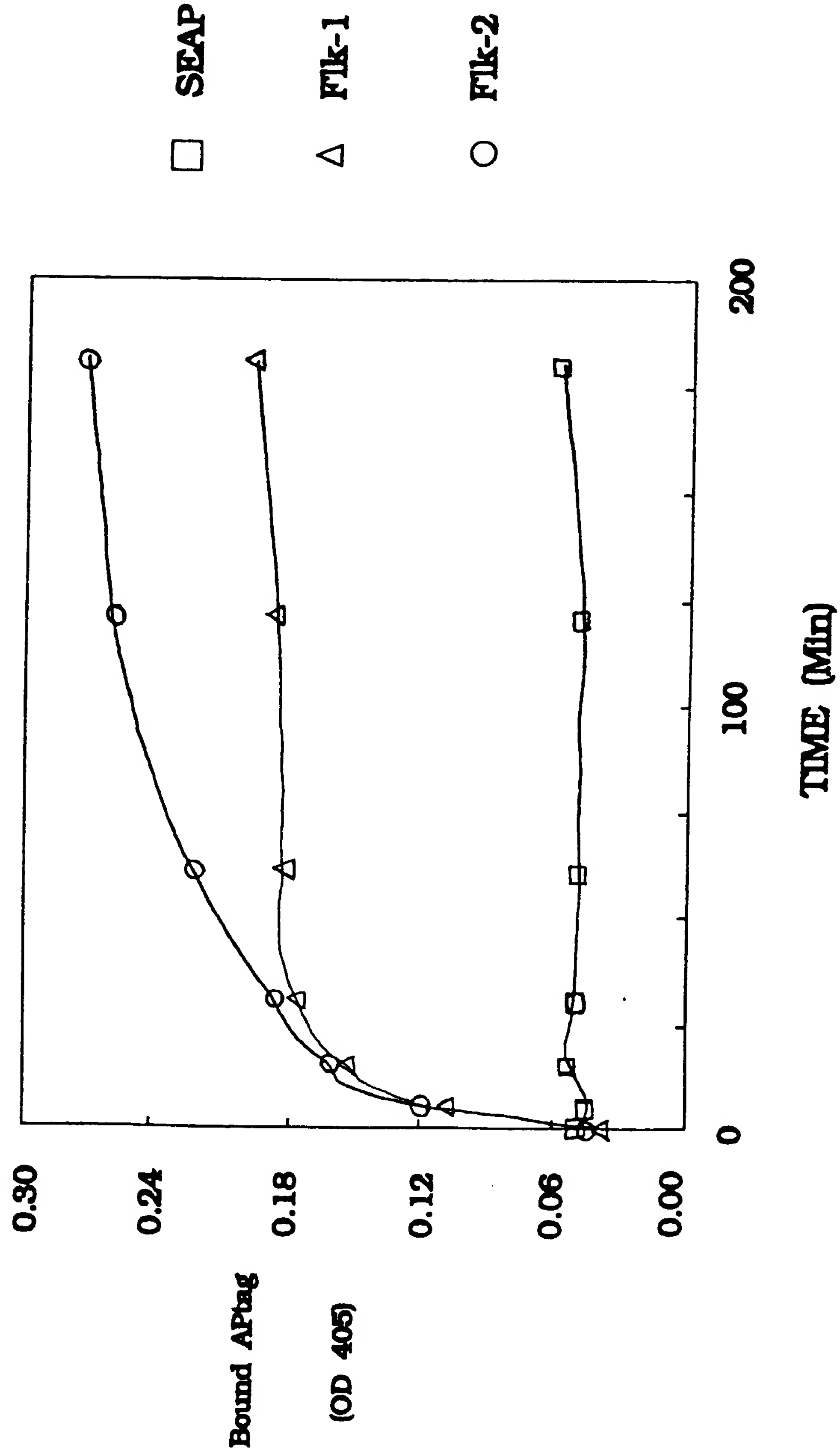
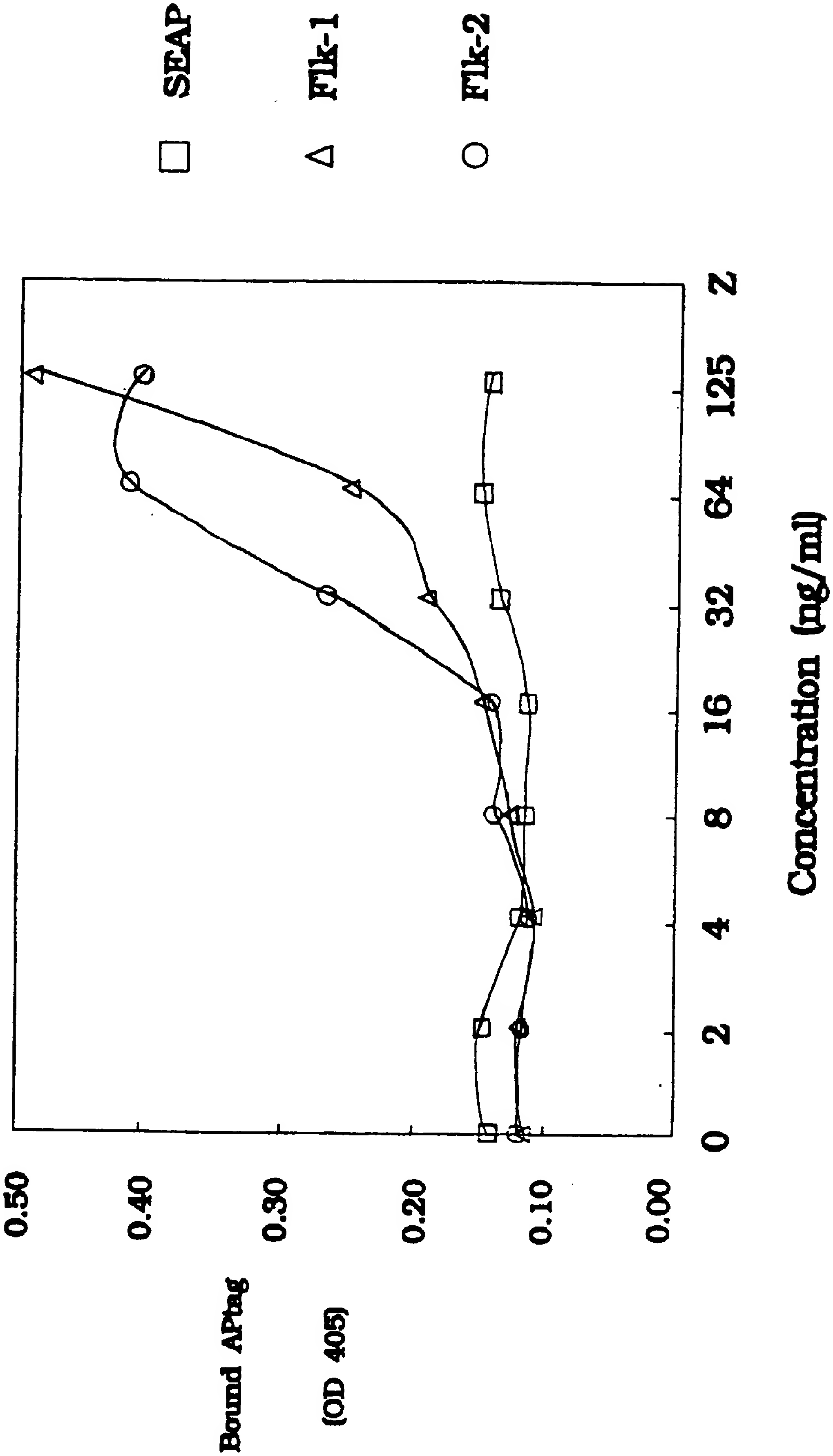

SEAP
Flk-1
Flk-2
0.30
0.24
0.18
0.12
0.06
0.00
Bound APtag
(OD 405)
0
100
200
TIME (Min)

FIGURE 4


SEAP
Flk-1
Flk-2
Bound APtag
(OD 405)
0.50
0.40
0.30
0.20
0.10
0.00
0
2
4
8
16
32
64
125
Z
Concentration (ng/ml)

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US92/02750

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): C07H 15/12, 17/00; A61K 37/00; C07K 13/00, 15/00; C12N 5/00
US CL : 536/27; 530/350, 387, 846; 514/2; 435/240.2

II. FIELDS SEARCHED

Minimum Documentation Searched[4]

Classification System	Classification Symbols
U.S.	536/27; 530/350, 387, 846; 514/2; 435/240.2

Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched[5]

Sequence Search: GENBANK, SWISS PROT, PIR, CAS
search terms: sequences of figures 1 and 2

III. DOCUMENTS CONSIDERED TO BE RELEVANT[14]

Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No. [18]
X,P Y	Proc. Nat. Acad. Sci. USA, Volume 88, issued October 1991, W. Matthews et al., "A receptor tyrosine kinase cDNA isolated from a population of enriched primitive hematopoietic cells and exhibiting close genetic linkage to *c-kit*", pages 9026-9030, see entire document.	1 4 - 17,19,22,25,28 ,31,34,40 1-13, 18, 20, 21,23,24,26,27 ,29,30,32,33,35-39,41-64
Y	Cell, Volume 63, issued 05 October 1990, J.G. Flanagan et al., "The *kit* ligand: a cell surface molecule altered in steel mutant fibroblasts", pages 185-194, see entire document.	41-64
Y	Proc. Nat. Acad. Sci. USA, Volume 86, issued March 1989, A.F. Wilks, "Two putative protein-tyrosine kinases identified by applicaton of the polymerase chain reaction", pages 1603-1607, see entire document.	1-64

* Special categories of cited documents:[15]

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search[2]	Date of Mailing of this International Search Report[2]
26 June 1992	07 JUL 1992
International Searching Authority[1]	**Signature of Authorized Officer**[20]
ISA/US	LORRAINE M. SPECTOR, PH.D.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No.[18]
A	Science, Volume 241, issued 01 July 1988, S.K. Hanks et al., "The protein kinase family: conserved features and deduced phylogeny of the catalytic domains", pages 42-52, see entire document.	1-64
Y	R. Hay et al., "American Type Culture Collection Catalogue of Cell Lines and Hybridomas, Fifth Edition", published 1985 by American Type Culture Collection (MD), see page 232.	65

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X,P Y	Cell, Volume 65, issued 28 June 1991, W. Matthews et al., "A receptor tyrosine kinase specific to hematopoietic stem and progenitor cell-enriched populations", pages 1143-1152, see entire document.	1 - 13,18,20,21,23 ,24,26,27,29,3 0,32,33,35 1 4 - 17,19,22,25,28 ,31,34,41,43,4 , 46-49, 53- 56,58-60,62,63

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE[1]

1. ☐ Claim numbers _, because they relate to subject matter (1) not required to be searched by this Authority, namely:

2. ☐ Claim numbers _, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out (1), specifically:

3. ☐ Claim numbers _, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING[2]

This International Searching Authority found multiple inventions in this international application as follows:

Please See Attached Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application. (Telephone Practice)
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Search Authority did not invite payment of any additional fee.

Remark on protest

☐ The additional search fees were accompanied by applicant's protest.

☒ No protest accompanied the payment of additional search fees.